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AVALIAÇÃO DA ATIVIDADE TRIPANOCIDA DA LECTINA DE *VATAIREA MACROCARPA* SOBRE A FORMA EVOLUTIVA TRIPOMASTIGOTA DO *TRYPANOSOMA CRUZI,* CEPA Y

FORTALEZA
2023

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Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Ceará, como requisito parcial à obtenção do título de Mestre em Bioquímica. Área de concentração: Bioquímica Vegetal.

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Aprovada em: 30/03/2023

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A Deus.

Aos meus pais, ao meu irmão, Xavier Santos.

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“Depois de todo esse tempo? Sempre...” (Harry Potter e as Relíquias da Morte - Parte 2, 2011).

RESUMO

A doença de Chagas, protozoose causada pelo *Trypanosoma cruzi*, acomete milhares de pessoas mundialmente. Classificada como doença tropical negligenciada, apresenta inúmeras dificuldades em seu enfrentamento, seja pela ausência de medicações específicas, tecnologias acessíveis ao diagnóstico ou programas efetivos de controle da transmissão. Nesse cenário, a pesquisa objetivou avaliar as tecnologias em saúde patenteadas para tratar e diagnosticar a doença, assim como verificar a atividade tripanocida da lectina de *Vatairea macrocarpa* – VML contra formas evolutivas tripomastigotas do *T. cruzi*, cepa Y. Para tanto, foi realizado um levantamento das patentes disponíveis nas bases de dados Cortellis Drug Discovery Intelligence (CDDI) e ESPACENET. A ação tripanocida da lectina VML foi testada quanto à sua citotoxicidade em células hospedeiras em cultura de células LLC-MK2, nas concentrações de 15,5 µg/mL, 31,2 µg/mL, 62,5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL e 1000 µg/mL. A lectina teve ainda sua ação testada na condição de inibição do domínio de reconhecimento a carboidrato por lactose. Para avaliar a afinidade da lectina pelos glicoinositolfosfolipídio (GIPL), foram realizados testes *in silico* a partir de ancoramentos moleculares. Inicialmente, os GIPL foram modelados no programa ChemSketch e otimizados no software Avogadro. A proteína foi recuperada do banco de dados Protein Data Bank. As simulações foram realizadas utilizando o servidor AutoDock Vina, para o qual o *grid box* foi estabelecido com as seguintes dimensões: center_x = -21.952, y = -19.851, z = -29.065 e size_x = 24, y = 22, size_z = 18. Ao final do estudo, foram identificadas 31 patentes referentes a moléculas candidatas a novos fármacos, com apenas duas delas em fase de testes clínicos, e 10 patentes referentes a técnicas de diagnóstico, sendo as universidades as principais solicitantes. Nos testes *in vitro*, a lectina apresentou índice de CC50 de 2744,00 ± 548,00 (> 1000); quando inibida por lactose, não foi possível estimar esse valor para as concentrações testadas. A lectina VML apresentou LC50 de 125,70 ± 22,70. O índice de seletividade da VML (IS) foi de 21,83, tornando-a um candidato a novos estudos. O ancoramento molecular apontou maior afinidade para o GIPL em relação ao sacarídeo Gal-GalNAc-GlcNAc: GIPL, -2,6 kcal/mol; Gal-GalNAc-GlcNAc, -3,5 kcal/mol; e lactose, -5,1 kcal/mol. Os dados demonstram que os sacarídeos presentes na estrutura se ligam ao domínio lectínico da proteína. A ação tripanocida identificada é promissora, e novas investigações que ajudem a compreender os mecanismos de ação envolvidos na atividade da lectina sobre o parasito são necessárias.

Palavras-chave: Prospecção tecnológica; Cepa Y; Atividade Tripanocida; GIPL.

ABSTRACT

Chagas disease, a protozoosis caused by *Trypanosoma cruzi*, affects thousands of people worldwide. Classified as a neglected tropical disease, it presents numerous challenges in its management, whether due to the lack of specific medications, accessible diagnostic technologies, or effective transmission control programs. In this scenario, the research aimed to evaluate patented health technologies for treating and diagnosing the disease, as well as to verify the trypanocidal activity of the *Vatairea macrocarpa* lectin (VML) against the trypomastigote evolutionary forms of *T. cruzi*, Y strain. To this end, a survey of available patents was conducted in the Cortellis Drug Discovery Intelligence (CDDI) and ESPACENET databases. The trypanocidal action of the VML lectin was tested for cytotoxicity in host cells using LLC-MK2 cell cultures at concentrations of 15.5 µg/mL, 31.2 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/mL, and 1000 µg/mL. Furthermore, the lectin's action was tested under conditions of carbohydrate-recognition domain (CRD) inhibition by lactose. To evaluate the lectin's affinity for glycoinositolphospholipids (GIPL), *in silico* tests were performed using molecular docking. Initially, the GIPLs were modeled in the ChemSketch program and optimized using Avogadro software. The protein was retrieved from the Protein Data Bank (PDB). Simulations were performed using the AutoDock Vina server, for which the grid box was established with the following dimensions: center_x = -21.952, y = -19.851, z = -29.065 and size_x = 24, y = 22, size_z = 18. At the end of the study, 31 patents regarding candidate molecules for new drugs were identified, with only two in the clinical trial phase, and 10 patents regarding diagnostic techniques, with universities being the primary applicants. In the *in vitro* tests, the lectin presented a CC_{50} 2744.00 ± 548.00; when inhibited by lactose, it was not possible to estimate this value for the tested concentrations. The VML lectin presented an LC_{50} 125.70 ± 22.70. The Selectivity Index (SI) of VML was 21.83, making it a candidate for further studies. Molecular docking indicated a higher affinity for GIPL compared to the saccharide Gal-GalNAc-GlcNAc: GIPL, -2.6 kcal/mol; Gal-GalNAc-GlcNAc, -3.5 kcal/mol; and lactose, -5.1 kcal/mol. The data demonstrate that the saccharides present in the structure bind to the lectin domain of the protein. The identified trypanocidal action is promising, and further investigations to help understand the mechanisms of action involved in the lectin's activity against the parasite are necessary.

Keywords: Strain Y; Technological prospecting; Trypanocidal action; GILP.

SUMÁRIO

1 INTRODUÇÃO

A Doença de Chagas (DC), uma parasitose endêmica das Américas e uma das doenças tropicais negligenciadas mais importantes do mundo, tem chamado atenção para a tendência de tornar-se uma epidemia global em razão dos movimentos migratórios (VELASCO; MURILLO, 2020; ECHAVARRÍA et al., 2021; HOCHBERG; MONTGOMERY, 2023). Diferentes fatores podem contribuir para esse fato, como a ausência de diagnósticos em determinados grupos populacionais, o acesso a tratamentos adequados, programas sanitários e a falta de educação em saúde pública e de investimentos em pesquisas na área (MILLS, 2020; EDWARDS; MONTGOMERY, 2021).

Enquanto epidemia, o combate à parasitose requer a eliminação do inseto vetor e a melhoria na qualidade das habitações das populações. Em alguns países, o problema vem sendo enfrentado de maneira adequada, como na Argentina, Chile, Uruguai e no Sul do Brasil. No entanto, no Brasil, a doença ainda é uma das causas de morte negligenciada em diversas regiões do país (MARTINS-MELO; CASTRO; WERNECK, 2021).

Apesar de campanhas bem-sucedidas em alguns países, grandes dificuldades são encontradas em outros como El Salvador, Honduras e Guatemala. Contudo, essa problemática de saúde não afeta apenas as economias em desenvolvimento. Estima-se que 240.000 a 350.000 pessoas residentes nos Estados Unidos estejam infectadas pelo *Trypanosoma cruzi*. A problemática é agravada pela dificuldade de acesso aos serviços de saúde e a cuidados eficazes, em especial no que se refere ao acesso a esses cuidados por imigrantes (HOCHBERG; MONTGOMERY, 2023; MILLS, 2020).

Nesse sentido, é urgente a necessidade do desenvolvimento de novas alternativas para intervir sobre esse problema. A esse respeito, diferentes estratégias vêm sendo pensadas, tais como tecnologias de diagnóstico (GYSIN et al., 2022), reposicionamento de fármacos (BELLERA et al., 2020) e a busca por novos princípios ativos (MAGALHÃES et al., 2022), assim como intervenções nas formas de tratar socialmente o tema com a população (LIU; CHEN; ZHOU, 2020).

Logo, as propostas de intervenção passam pela implementação de novas tecnologias de cuidado em saúde e pelo monitoramento do surgimento de novos casos. O presente documento aborda o mapeamento das tecnologias de saúde patenteadas sobre o tema e a avaliação da ação tripanocida de uma lectina ligante á galactose, extraída das sementes de *Vatairea macrocarpa*.

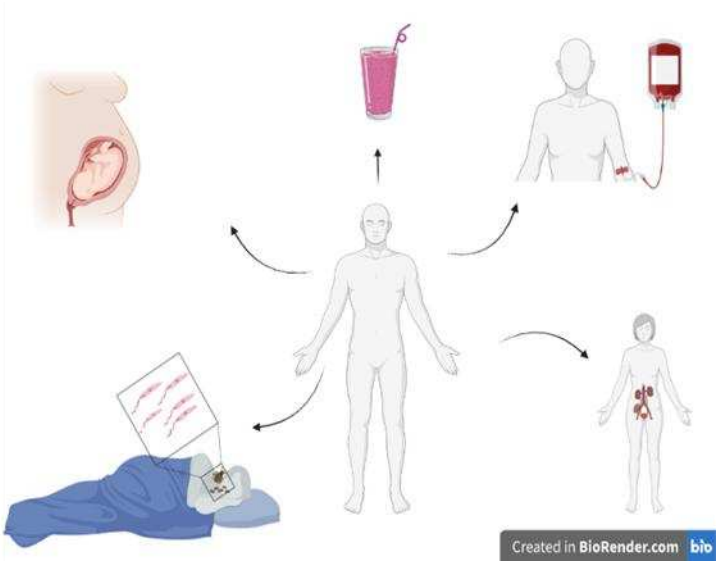
1.1 Doença de Chagas

A doença de Chagas, descrita no ano de 1909 pelo pesquisador e médico Carlos Justiniano Ribeiro Chagas teve seu agente etiológico identificado incialmente em 1908 em amostra de sangue de macacos da espécie *Callitrix penicillata* comum na região Norte de Minas Gerais, o parasito denominado como *Trypanossoma cruzi*. Posteriormente, ao analisar o componente estomacal de besouros hematófagos da espécie *Panstrongylus megistus*, vastamente encontrado em casas na região, foi observado a presença do mesmo parasita flagelado, sendo então encaminhado para o médico sanitarista Oswaldo Cruz para complementar as análises e a confirmação da identificação previamente realizada por ele. EM seu trabalho, Carlos Chagas conseguiu ainda realizar a descrição do ciclo de vida do parasito e estabelecer os reservatórios silvestres para o *T. cruzi*, o tatu (*Dasypus novemcinctus*), assim como, a identificação do primeiro registro de infecção em humanos, o caso Berenice, para a qual foi descrita as manifestações clínicas referente à fase aguda da doença em 1909 (COURA, 1997).

As manifestações clínicas da Doença de Chagas distinguem-se nas diferentes fases da infecção. A fase aguda é marcada pela elevação do número de parasitos no hospedeiro, situação na qual é possível observar sintomas como febre, mal-estar, falta de apetite, edemas, sinal de Romaña (caracterizado pelo inchaço da pálpebra), aumento do baço e do fígado e distúrbios cardíacos. Em crianças, o quadro pode se agravar e levar à morte. A fase indeterminada é geralmente assintomática; e a fase crônica pode ser caracterizada pela ausência de sintomas, apesar de o indivíduo portar o parasito. Nessa fase, é possível haver comprometimento causado pelo aumento de órgãos, como o coração e o aparelho digestivo, classificando-a como a doença dos megas. (PÉREZ-MOLINA; MOLINA, 2018; CORREIA, et al., 2021; SOUZA, 2019).

Diferentes formas de transmissão foram descritas para o agente etiológico da DC, dentre elas: transfusão de sangue ou hemoderivados de doadores infectados (CROWDER, 2022); congênita (de mãe para filho) durante a gravidez ou o parto (CARLIER; TRUYENS; MURAILLE, 2021); por transplante de órgãos de doadores infectados (CAMERA et al., 2018); ou ainda o consumo de alimentos contaminados (FERREIRA; BRANQUINHO; LEITE, 2014), como ilustrado na Figura 1.

Figura 1 – Formas de transmissão do *Tripanossoma cruzi.*



Fonte: Elaborado pelo autor.

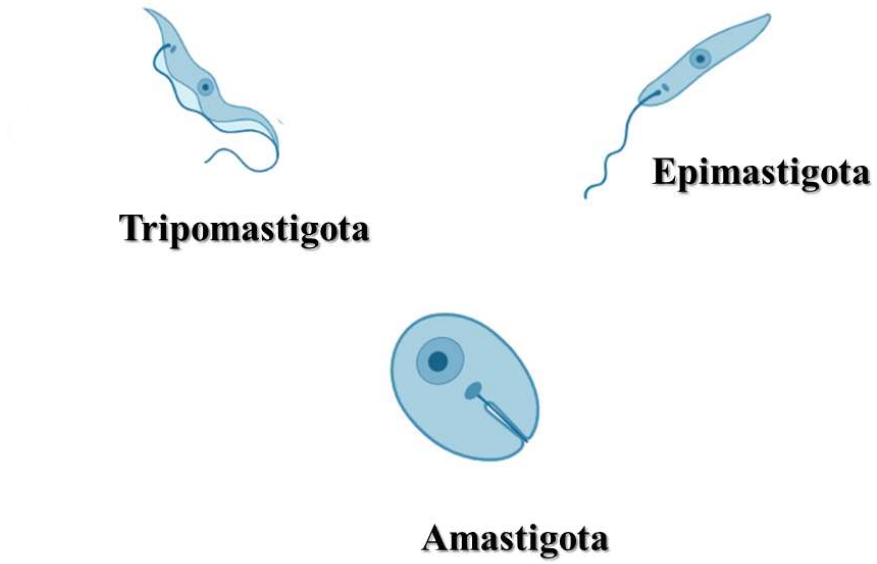
Hoje, diversas técnicas são empregadas para a determinação do diagnóstico do *T. cruzi*, podendo este ser realizado de acordo com a fase da doença. Para o estabelecimento do diagnóstico durante a fase aguda, os testes realizados consistem na utilização de sangue a fresco, por meio de esfregaço e gota espessa. Já para a fase crônica, em razão do declínio na carga parasitária, são empregados métodos parasitológicos indiretos, sendo eles o xenodiagnóstico ou o hemocultivo. Os testes sorológicos envolvem testes de elevada sensibilidade, como o teste de ELISA, os quais utilizam o antígeno total ou frações semipurificadas do parasito; outra possibilidade é a utilização de antígenos recombinantes específicos para o *T. cruzi*. Em alguns casos, a técnica da Reação em Cadeia da Polimerase (PCR) pode ser utilizada para a detecção do DNA presente no cinetoplasto (kDNA) (LIMA; TEIXEIRA; LIMA, 2018).

Com os avanços nas tecnologias para o diagnóstico e monitoramento da epidemiologia da doença de Chagas, foi iniciada, então, uma ação de controle do vetor em 1980, por meio de políticas de saúde que levaram ao monitoramento e controle das vias de transmissão por transfusão sanguínea (CORRASA et al., 2016). Desse modo, avanços foram observados na modificação do perfil e na redução de casos, agora com prevalência em sujeitos com baixa escolaridade e o predomínio da transmissão de forma vetorial, seguido da diminuição do aparecimento de novos casos (HASSLOCHER-MORENO et al., 2021).

1. 2 *Trypanosoma cruzi*

O agente etiológico da DC, *T. cruzi*, pertence ao grupo dos flagelados e a ordem Kinetoplastida, classificado como pertencente ao supergrupo dos Excavata, e a Divisão dos Euglenozoa (ADL et al., 2005). Caracterizado por apresentar um ou dois flagelos e uma única mitocôndria, na qual seu Ácido Desoxirribonucleico – DNA encontra-se condensado próximo a região basal do flagelo, denominado cinetoplasto, de onde deriva o nome do grupo (NEVES, 2005). A morfologia celular do *T. cruzi* encontra-se sintetizada na figura 2.

Figura 2 – Formas celulares encontradas durante a infecção do *Trypanosoma cruzi*.

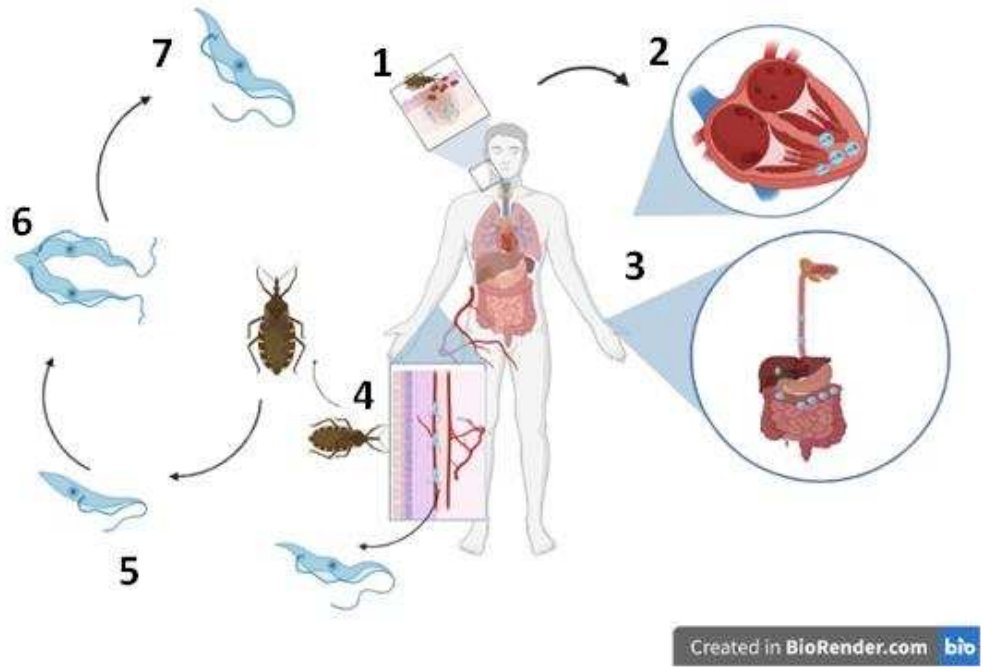


Fonte. Elaborado pelos autores.

Inicialmente identificado pelo cientista brasileiro Carlos Chagas durante um trabalho de controle da malária em Minas Gerais, o pesquisador identificou um protozoário com grande cinetoplasto e intensa movimentação, que encaminhou para identificação no Rio de Janeiro pelo pesquisador Oswaldo Cruz como sendo um parasito novo. Posteriormente, em 1909, Carlos Chagas identificou-o no sangue de uma criança de 2 anos, Berenice, apresentando o mesmo quadro clínico observado em animais de laboratório infectados pelo protozoário. Desse modo, o pesquisador conseguiu descrever a biologia e a morfologia do parasito no hospedeiro vertebrado, assim como no hospedeiro invertebrado. O trabalho realizado pelo pesquisador foi responsável por descrever a sintomatologia da doença durante a fase aguda, assim como os reservatórios naturais para a espécie (CHAO; LEONE; VIGLIANO, 2020).

Caracterizado como um parasito heteróxeno, seu ciclo de vida é completado através da infecção de um hospedeiro vertebrado (homem e outros mamíferos), momento no qual ocorre sua multiplicação intracelular, e na infecção de um hospedeiro invertebrado do gênero *Triatoma*, com sua reprodução de modo extracelular. Diferentes modificações morfológicas são observadas durante os estágios evolutivos no parasito, marcados pela presença de três estágios principais (DE SOUZA, 1999; NEVES, 2005). O ciclo de vida do *T. cruzi* é sumarizado na figura 3, abaixo.

Figura 3 – Ciclo de vida do *Trypanosoma cruzi*



Fonte: elaborado pelo autor.

1. Repasto sanguíneo e eliminação de fezes do inseto triatomíneo contendo *Trypanosoma cruzi* em formas evolutivas tripomastigotas; esses adentram a corrente sanguínea através de ferimentos na pele; 2. Infecção de células e diferenciação em formas intracelulares, amastigotas; 3. Amastigotas iniciam um acentuado processo de reprodução por divisão binária, voltando a se diferenciar em tripomastigotas; 4. Liberados na corrente sanguínea, infectam outros tecidos em um ciclo comtínuo de infecção; 5. Ao realizar o repasto sanguíneo em um hospedeiro infectado, o parasito invade as células do intestino médio do inseto e transforma-se em epimastigotas; 6. Início de um novo ciclo de reprodução por divisão binária; 7. Migração para o intestino posterior seguida de diferenciação em formas tripomastigotas, onde estarão aptos a um novo processo de infecção.

Atualmente as cepas do *T. cruzi* estão classificadas segundo seu nível de virulência e patogenicidade apresentadas em hospedeiro vertebrados, são elas, cepas com baixa virulência e patogenicidade (AM64/TcIV e 3253/Tc-V), média virulência e patogenicidade (PL1.10.14/TcIII e CL/TcVI) e alta virulência e patogenicidade (Colombiana/Tc-I e Y/TcII)

pertencentes às seis unidades de tipagem discretas (DTUs[1])—TcI a TcVI (WILLIAM et al., 2006), (JANSEN et al., 2020). A diversidade presente no grupo referente as variações biológicas, imunológicas e de resistência a intervenção farmacológica é acentuada entre os diferentes indivíduos, sendo possível considerar o grupo como um complexo de espécies (COURA et al., 1966).

Contudo, nesse estudo é investigada a cepa Y, considerada de alta relevância, pois apresenta elevado grau de virulência e resistência aos fármacos disponíveis para tratar a doença de Chagas. Inicialmente identificada em 1953 em uma paciente de origem japonesa residente em Marília, no estado de São Paulo, a cepa coletada do sangue da paciente foi encaminhada para caracterização pelo Departamento de Parasitologia da Faculdade de Medicina de São Paulo, o qual identificou alta mortalidade em animais de laboratório infectados pelo parasito colhido da paciente. Tal característica peculiar rendeu a denominação de cepa Y em referência à paciente da qual foi obtido o parasito; desde então, diversas pesquisas foram realizadas utilizando-a como modelo (AMATO NETO, 2010).

A agressividade observada na cepa a tornou modelo para diferentes investigações, entre elas, as que buscam compreender o mecanismo que concede tal avidez em escapar do sistema imune. É sabido inicialmente que o acréscimo de glicosilação auxilia no escape ao recobrir epítopos presentes na membrana celular do parasito (LEDERKREMER; AGUSTI, 2009). Desse modo, o perfil glicídico presente nas superfícies de membranas do parasito pode ser alvo de intervenção farmacêutica (SINGH et al., 1994).

No que se refere à glicosilação das membranas, um conjunto de estruturas complexas já foi identificado envolvendo carboidratos, lipídios e proteínas, demonstrando haver diferenças nos tipos de carboidratos e lipídios que os ancoram nas membranas (FERGUSON, 1999). A variação é observada também em diferentes estágios de desenvolvimento do parasito, com a predominância de determinados tipos de carboidratos entre os estágios.

1. 3 Glicobiologia do *Trypanosoma cruzi*: diversidade de glicanos e alvos moleculares

Entre as diversas estratégias no desenvolvimento de tecnologias aplicadas ao tratamento da doença de Chagas, existe um segmento de pesquisas que exploram os aspectos da glicobiologia desse parasito. Nessa perspectiva, alguns trabalhos (PEREIRA-CHIOCCOLA,

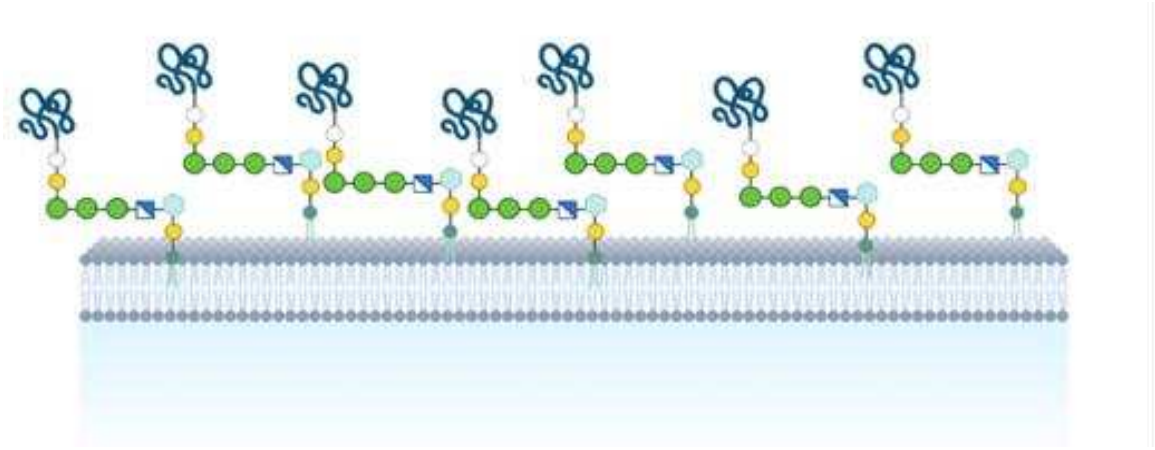
[1] Unidades de Tipagem Discreta (DTU) pode ser compreendida como um conjunto de populações semelhantes geneticamente, passiveis de serem identificadas através de padrões genético, imunológicos e moleculares comum ao grupo.

2000; FERGUSON, 1997; SINGH et al., 1994; LEDERKREMER; AGUSTI, 2009) foram desenvolvidos no sentido de identificar e caracterizar os tipos de glicanos presentes nas membranas celulares do *T. cruzi*.

O primeiro grupo de glicanos identificados no parasito foram os glicoinositolfosfolipídios (GIPL), abundantemente distribuídos nas superfícies celulares de epimastigotas e tripomastigotas metacíclicos. Apesar de identificados nas duas formas, a presença de GIPL é maior nas formas evolutivas de epimastigotas (LEDERKREMER; AGUSTI, 2009). A composição de açúcares nesses glicanos é diversificada, assim como os lipídios presentes.

Investigações voltadas para a determinação da composição desses glicanos permitiu identificar as seguintes sequências de glicanos: bGalf-(1→3)-aMan-(1→2)-aMan-(1→6)-aMan-(1→4)-aGlcN-(1→6)-myoIno-1-P-ceramide AEP; bGalf -(1→3) bGalf-(1→3)-aMan-(1→2)-aMan-(1→2)-aMan-(1→6)-aMan-(1→4)-aGlcN-(1→6)-myoIno-1-P-ceramide 65%) AEP (20%) AEP; bGalf-(1→3)-aMan-(1→2)-aMan-(1→2)-aMan-(1→6)-aMan-(1→4)-aGlcN-(1→6)-myoIno-1-P-ceramide (FERGUSON et al. 1988).

Figura 4**.** Padrão de glicosilação da superfície celular das formas evolutivas epimastigotas, cepa Y, *Trypanosoma cruzy*.



Fonte: elaborado pelo autor.

Parte desses glicanos encontram-se associados a proteínas da superfície celular, mucinas, desempenhando inúmeros atividades desde escape imunológico a aumento da infectividade do parasito (PEREIRA-CHIOCCOLA, et al., 2020). Além das mucinas, as Trans-sialidase, família de glicoproteínas, desempenhos outras atividades biológicas. Para essas, foi observado a substituição do núcleo trimanosil por uma α-galactose (LEDERKREMER; AGUSTI, 2009)

Ao analisar a composição dos glicanos presentes nas formas evolutivas epimastigotas do *T. cruzi* foi determinado o seguinte perfil de monossacarídeos galactose, glucosamina, manose, ácido siálico e vestígios de galactosamina e Inositol (SINGH, et al.., 1994). Ainda segundo os autores o perfil de glicanos encontrado no *T. cruzi* é significativamente maior que o encontrado em *Leshimania*.

Tomando por base o perfil de glicanos e o papel por eles desempenhados no sucesso da infecção e disseminação do parasito, agentes de ligação a carboidratos têm sido considerado como estratégia para o desenvolvimento de novas drogas (VALENTE, et al., 2019). Contudo, a toxidade de lectinas e peptídeos pode ser considerado como obstáculo (VALENTE, et al., 2019). Dado a disponibilidade de agentes de reconhecimento de galactose, é possível avaliar a efetividades de lectinas ligantes a galactose e explorar o sacarídeo Galα(1,3)Galβ(1,4)GlcNAcα presente nas formas tripomastigotas (SCHOCKER, et al., 2016)

Contudo um desafio é posto, a ação das trans-sialidase que são responsáveis por transferir resíduos de ácido siálico para os resíduos de β-galactopiranosil presentes nas superfícies celulares do parasito, (FREIRE-DE-LIMA, et al., 2015), tornando indisponível parte das moléculas alvos das lectinas. Essa ação é efetuada pela doação de glicoconjugados dos hospedeiros e sua transferência para a superfície de mucinas do parasito modificando sua assinatura imunológica (CAMPETELLA, et al., 2020), podendo levar a redução da atividade das lectinas ligante a galactose.

1.4 *Vataeria macrocarpa*

Vataeria macrocarpa, uma leguminosa, conhecida popularmente como amargo, amargoso, pau roxo, sucupira preta. Do ponto de vista da taxonômico, a espécie pertence ao Domínio eukarya, Viridiplantae, Streptophyta, Magnoliopsida, família Fabaceae, subfamília Papilionodeae; Tribo Dalbergieae DC; Gênero: *Vatairea*, espécie *Vataeria macrocarpa* (Benth Ducke), (SCHOCH ET AL., 2020, ROYAL BOTANIC GARDENS, KEW, 2020).

Espécie é nativa do Brasil, e sua distribuição é observada desde a Bolívia (ROYAL BOTANIC GARDENS, KEW, 2020. A *V. macrocarpa* possui porte arbóreo com ampla distribuição no território nacional. Atualmente a espécie é descrita nos domínios fitogeográficos Amazônico, da Caatinga e do Cerrado, (Cerrado *lato sensu*). A espécie apresenta entre 5-12 metros de altura com folhas do tipo folioladas entre 5 e 9 folíolos. As flores são hermafroditas, com corres roxas e seu fruto é do tipo sâmara com sementes pouco carnosas (COSTA; LIMA, SILVA, 2014; CARDOSO; RAMOS; LIMA, 2022) como ilustrado na figura 5. A floração na

espécie ocorre entre o período de seca e sua frutificação entre o período de seca e da quadra chuvosa (FIGUEIREDO, 2008). A espécie é semidecídua, com período de frutificação bianual para alguns indivíduos. Estudos fenológicos envolvendo a espécie demonstraram padrão de floração não uniformizado para a espécie (COSTA, LIMA; SILVA, 2014).

Figura 5 – *Vatairea macrocarpa* (Benth.) Ducke

Fonte: árvore do Bioma Cerrado.
https://www.arvoresdobiomacerrado.com.br/site/2019/09/03/vatairea-macrocarpa-benth-ducke/

Estudos etnobotânicos tem indicado o uso medicinal de extratos produzidos a partir de partes da planta por populações tradicionais. Diferentes, aplicações como atividades antiúlceras e anti-inflamatórias, algumas delas já investigadas e comprovadas por estudos experimentais a partir de moléculas como o ácido crisofânico e 7-hidroxiflavona do cerne (MATOS et al., 1988) e a purificação de lectina das sementes (TEIXEIRA et al., 2006; ALENCAR et al., 2004, 2007). Contudo, os estudos envolvendo a espécie ainda são incipientes para comprovar a eficácia descrita pelos conhecimentos populares a ela atribuídos (JESUS, et al. 2009).

1.5 Lectina de *Vatairea macrocarpa*

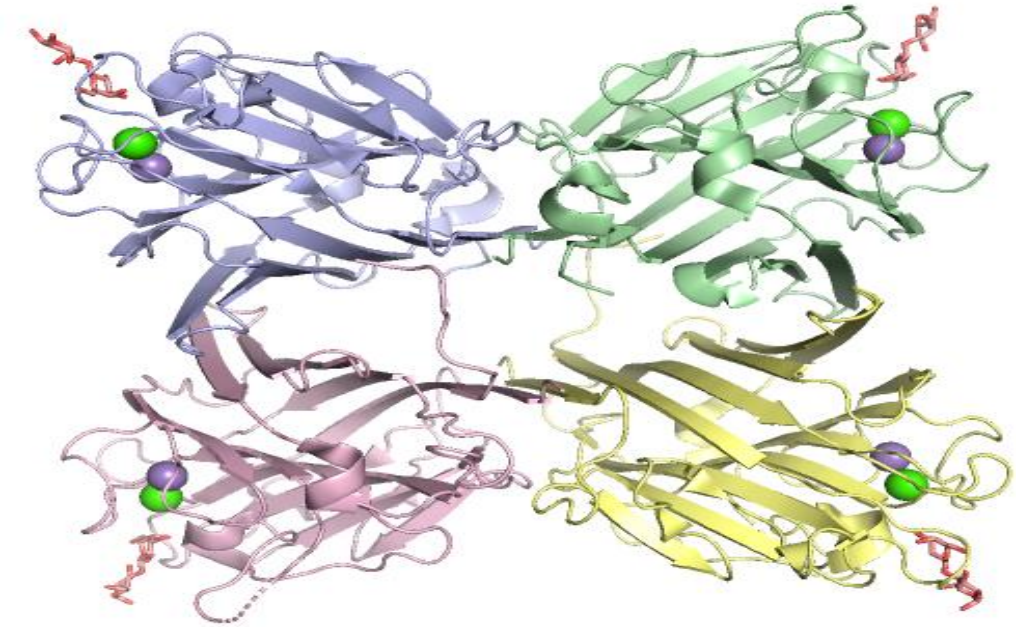
As lectinas correspondem a um grupo de proteínas com habilidade de ligação seletiva a carboidratos (HENDRICKSON; ZHERDEV, 2018). Esse grupo de proteínas

apresenta a capacidade de ligar-se a carboidratos e não modificar os carboidratos com os quais esteja interagindo (RÜDIGER; GABIUS, 2001).

Esse grupo de biomoléculas pode ser encontrado nos mais diferentes grupos de seres vivos e suas aplicações biotecnológicas são diversas. Diversos estudos têm mostrado que as lectinas podem apresentar aplicações variadas; dentre elas, já foram descritas a capacidade de aglutinação e promoção de atividades mitóticas (CARVALHO et al., 2018), alteração de atividades enzimáticas (SHARMA; GOKHALE, 2014), inibição do crescimento bacteriano (SILVA et al., 2019), ação antifúngica (KLAFKE et al., 2013), agregação celular (IURISCI, 2009), toxicidade (SIMPSON et al., 2018) e imunomodulação (MISHRA et al., 2019).

A lectina em estudo é extraída a partir das sementes de *V. macrocarpa*, VML, uma lectina de massa molecular estimada entre 122 e 130 kDa (CALVETE et al., 1988), com sua estrutura primária constituída por 240 resíduos de aminoácidos (CAVADA, 1998). Análises realizadas a partir de eletroforese em gel de poliacrilamida – SDS revelam três bandas com massas de 32-33 kDa referente a uma banda maior, cadeia α, e duas bandas menores de 22 e 13 kDa correspondentes às cadeias β e γ respectivamente, como indicado na Figura 4 (CAVADA et al., 1998; CALVETE et al., 1988). PDB – 4U36, 4WV8. A estrutura quaternária da proteína é um homotetrâmero, sendo sua estrutura constituída principalmente por folhas β em um total de 21 folhas e 3 α-hélices intercaladas por regiões desordenadas, *loops*.

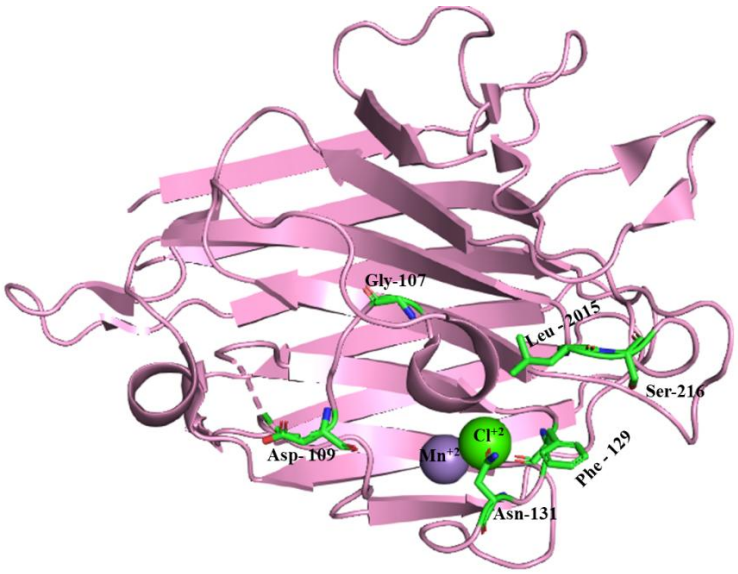
Figura 6 – Estrutura quaternária da Lectina de Vatairea macrocarpa – VML.



Fonte: PDB – 4U36.

Com afinidade por galactose e N-acetil-galactosamina (RAMOS, 2000) a proteína é capaz de reconhecer o antígeno T (DAM, 2007). A VML é uma metaloproteína cuja sua estrutura possui dois íons divalentes, Cálcio (Ca^{2+}) e Manganês (Mn^{2+}) por monômero. Em sua estrutura foi identificado um único Domínio de Reconhecimento a Carboidratos (DRC) envolvendo os resíduos Asp-109, Gly-107, Asn-131, Phe-129, Leu-215, Ser-216 (SOUSA, et al. 2016), como demonstrado na figura 7.

Figura 7 – Domínio de Reconhecimento a Carboidrato da Lectina de *Vatairea macrocarpa* - (VML).



Fonte: Elaborado pelo Autor – PDB 4U36. O domínio de reconhecimento a carboidratos da proteína é destaca pela porção em verde na figura. Destaque os íons manganês Mn^{+2} (em roxo) e Cloro Cl^{+2} (em verde) PDB: 4U36.

Em sua estrutura foi observado a presença de glicosilação do tipo N-glicosilado nos resíduos Asn-111 e Asn-183 com o heptassacarídeo [(beta-xilosil-1,2)(alfa-manosil-1,6)(alfa-manosil-1,3)]beta-manosil-1,4-GlcNAC-beta-1,4-GlcNAc-beta-1,4[alfafucosil-1,3]GlcNA (CALVETE et al, 1998). A proteína resiste a faixa de pH entre 2,5 e 8,5 (CALVETE et al, 1998).

Dado ao potencial já identificado para o uso de lectinas como ferramentas biotecnológicas para enfrentar problemas na saúde e na agricultura (MARTÍNEZ et al., 2004, GONÇALVES et al., 2013). O uso da lectina VML vêm sendo avaliadas para diferentes fins, como sumarizado no quadro - 1.

Quadro 1 - Atividades biológicas identificadas para a lectina de *Vatairea macrocarpa* VML

Atividade	**Modelo**	**Tipo de estudo**	**Autores**
Estimulação ao efluxo líquido específico de H+	*Rhizobium tropici*	*In vitro*	Martínez et al., 2004
Ação anti-biofilme e Redução da capacidade de adesão das bactérias	*Streptococcus mutans, S. sobrinus, S. sanguis, S. mitis, S. oralis*	*In vitro*	Texeira et al., 2005
Redução do crescimento de colônias de bacterias	*Staphylococcus aureus; S. epidermidis Pseudomonas aeruginosa, Candida albicans*	*In vitro*	Vasconcelos et al., 2014
Estímulo no crescimento de rizóbios	*Rhizobium tropici*	*In vitro*	Vasconcelos et al., 2013
Ausência de ação anticonceptiva orofacial	*Danio rerio*	*In vivo In silico*	Leite et al., 2020
Atividade citotóxico para linfócitos e genotóxico em concentrações superiores a (8 mM) e atividade antigenotóxica em concentrações inferiores a (0,5 e 2 mM) Atividade angiogênico e efeito pró-inflamatório	linfócitos humano e embriões de *Gallus domesticus*	*In vitro In vivo*	Veras et al., 2021
Capacidade de aumentar a perfusão da pressão arterial, resistência vascular renal, fluxo urinário e taxa de filtração glomerular.	Wistar rats	*In vivo*	Martins et al., 2005
Promoção de resposta inflamatória e migração de neutrófilos	Wistar rats were	*In vivo*	Alencar et al., 2003
Capacidade de potenciar a ação antibiótica da lectina quando em combinação com os antibióticos como gentamicina, norfloxacina e penicilina.	Staphylococcus aureus e Escherichia coli.	*In vitro*	Santos et al., 2019
Promoção de resposta neuroinflamatória; Ação no hipocampo de camundongo e com exibição de atividade depressiva.	Camundongos suíços albinos	*In vivo*	Gonçalves et al., 2013
Efeito pró-inflamatório dependente na presença de leucócitos.	Wistar rats	*In vivo*	Alencar et al., 2004
Capacidade de promover a migração de neutrófilos	Wistar rats	*In vitro in vivo*	Alencar et al., 2007

Fonte: elaborado pelo autor.

Aqui é avaliado o uso da lectina contra o parasito da DC, como estratégia para enfrentamento de uma questão de saúde pública nas Américas e na África.

2. OBJETIVOS

2.1 Objetivo Geral

Identificar as tecnologias de saúde disponível para o enfrentamento da doença de Chagas, assim como avaliar o efeito tripanocida da lectina de *Vatairea macrocarpa* – VML sob formas resistentes de *Trypanossoma cruzi*, cepa Y.

2.2 Objetivos específicos

- Inventariar as tecnologias da saúde patenteadas para tratar e diagnosticar a doença.
- Avaliar a citotoxicidade da lectina VML sob células hospedeira;
- Verificar o potencial tripanocida da lectina VML sobre formas tripomastigota de *T. cruzi*;
- Analisar se a ação da lectina ocorre pelo Domínio de Reconhecimento a Carboidrato – DRC;
- Identificar possíveis interações entre a lectina VML e os Glicoinositolfosfolípidios (GIPL) presentes na membrana plasmática do *T. cruzi*.

3 RESULTADOS E DISCUSSÃO

3.1 CAPÍTULO 1: HEALTH TECHNOLOGY APPLIED IN THE TREATMENT OF CHAGAS DISEASE: DRUGS AND DIAGNOSTIC METHODS

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[3,1]Lana Grasiela Alves Marques and Bruno Anderson Matias da Rocha have contributed equally

INTRODUCTION

Neglected tropical diseases (NTDs) correspond to a set of diseases caused by infectious agents or parasites that affect populations in tropical regions such as Africa, Asia and Latin America, especially vulnerable populations. Among this set of diseases we have Chagas disease, leishmaniasis, schistosomiasis, among many others. This denomination stems from the low investment in health technologies for diagnosis and treatment, especially since it is not a priority on the global agenda (Engels and Zhou, 2020; World Health Organization, 2010).

Among this group of diseases, it is estimated that between 6 and 7 million people are infected by *T. cruzi*, the etiological agent of Chagas disease (CD), with the prospect that a population of 75 million are at risk of infection when considered factors such as lack of diagnoses, treatments and permanence of populations in remaining transmission areas (Chagas disease - American trypanosomiasis). Approximately 12,000 deaths due to CD are recorded annually (Thakare et al., 2021).

(Bartsch et al., 2018.

Currently, only two drugs, nifurtimox and benznidazole, are used to treat the acute phase of the disease (Thakare et al., 2021; Kratz, 2019). The drug events observed during treatment are responsible for the discontinuation of medication use (Jackson et al., 2020). Among the reported adverse effects, mucocutaneous symptoms were described for benznidazole and digestive symptoms for nifurtimox. Neuropsychiatric effects in both groups (Jackson et al., 2020). Therefore, the urgency and need for prospecting for new molecules that can be incorporated into the treatment of CD is evident.

Described in 1909 by researcher and physician Carlos Justiniano Ribeiro Chagas, CD

had its etiologic agent initially identified in 1908, the parasite known as *T. cruzi*. The first documented record of infection in humans, the Berenice case, occurred in 1909, when clinical manifestations related to the acute phase of the disease were described (Rodrigues Coura, 1997).

Clinical manifestations in CD are distinguished in the different phases of the disease infection, during the acute phase, marked by the increase in the number of parasites in the host, it is possible to observe symptoms such as fever, malaise, lack of appetite, edema (Roman sign), characterized as swelling of the eyelid, enlargement of the spleen and liver and heart disorders. In children, the condition can get worse and lead to death. The undetectable phase, usually asymptomatic; the acute phase can be characterized by the absence of symptoms, despite carrying the parasite, and there may be compromised growth in organs such as the heart and digestive system, classifying it as a chronic disease (Souza and Souza, 2019; Correia et al., 2021;)

Since the first case was reported, different forms of transmission have been described for the contagion, including blood transfusion or blood products from infected donors (Crowder et al., 2022), congenital (from mother to child) during pregnancy or childbirth (Carlier et al., 2021); by organ transplantation from infected donors (Pierrotti et al., 2018) or consumption of contaminated food (Barroso Ferreira et al., 2014).

Today, some techniques are used to diagnose *T. cruzi*. These are carried out according to the stage of the disease, for the acute stage the tests carried out consist of fresh blood, through smears and thick blood smears. As for the diagnosis in the chronic phase, due to the decline in the parasitic load, indirect parasitological methods are used, such as xenodiagnosis or blood culture. Serological tests involve the highly sensitive Enzyme Linked ImmunonoSorbent Assay (ELISA) tests, which use the total antigen or semi-purified fractions of the parasite, another possibility is the use of specific recombinant antigens for *T. cruzi*. In some cases, the Polymerase Chain Reaction (PCR) technique can be used to detect the DNA present in the kinetoplast (kDNA) (Lima et al., 2019).

With the advances made about Chagas disease, a vector control policy was initiated in 1980 through health policies that led to vector control strategies, monitoring and control of transmission routes by blood transfusion (Bello Corassa et al., 2016). Advances were observed from the modification of the profile and reduction of cases with prevalence of cases in subjects with low education and the predominance of vector transmission followed by the decrease in the appearance of new cases (Hasslocher-Moreno et al., 2021).

The parasite, *T. cruzi*, belongs to the flagellate group and the Kinetoplastida order. Characterized by having one or two flagella and a single mitochondrion, in which its

Deoxyribonucleic Acid - DNA is found condensed near the basal region of the flagellum, called kinetoplast, from which the name of the group derives (de Souza, 1999). The cellular morphology of *T. cruzi* is summarized in figure 1.

Characterized as a heteroxenous parasite, its life cycle is complex and begins with the infection of a vertebrate host (man and other mammals), in which intracellular multiplication occurs, and with the infection of an invertebrate host of the genus Triatoma, where reproduction occurs. intracellularly (Tyler and Engman, 2001), the lifecycle is summarized in the figure below, figure 1.

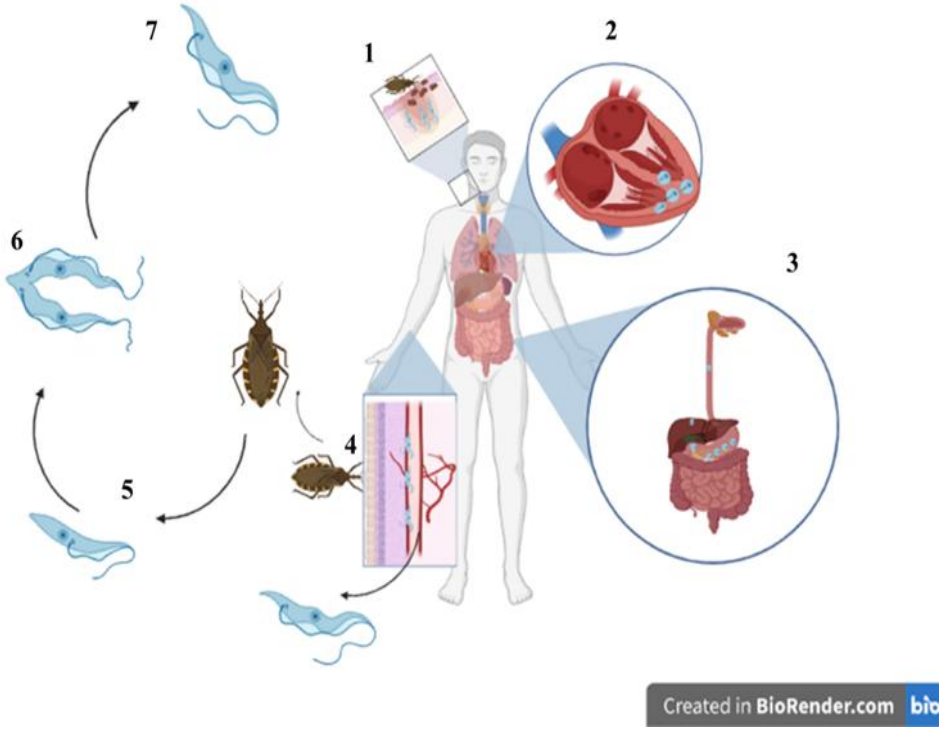


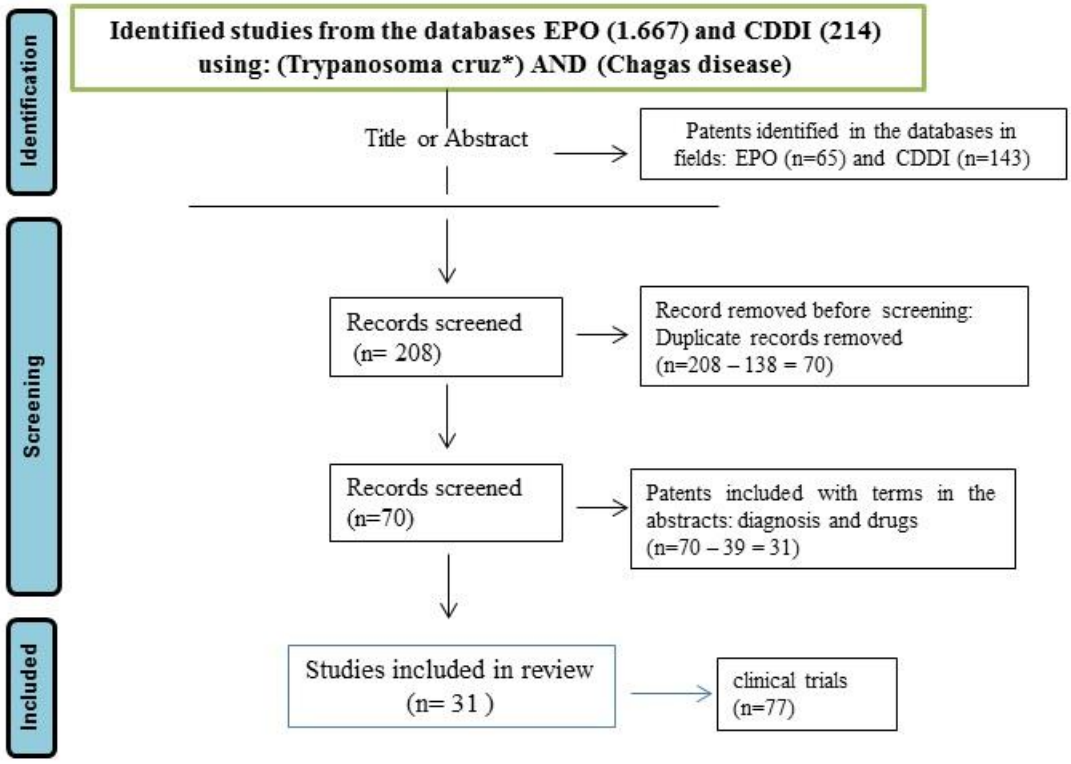
Figure 1. Chagas disease: morphology and life cycle of *Trypanosoma cruzi.*
1. Blood meal and elimination of feces of the triatomine insect infected by *T. cruzi* in trypomastigotes evolutionary forms, these enter the bloodstream through wounds in the skin; **2.** Infection of cells and differentiation into intracellular forms, amastigotes; **3.** Amastigotes initiate a marked process of reproduction by binary division, re-differentiating into Trypomastigotes; **4.** Released from Trypomasthiota forms in the blood stream and infection of other tissues in a continuous cycle of infection; **5.** When performing a blood meal on an infected host, the parasite invades the midgut cells of the insect and transforms into epimastigotes; **6.** Beginning of a new reproduction cycle by binary division; **7.** Migration to the hindgut followed by differentiation into trypomastigotes, where they will be able to undergo a new infection process.

Based on knowledge about the parasite's morphology and molecular mechanisms, different strategies to treat the disease have been developed, either in the creation of new drugs or in ways of diagnosing the disease. With a view to contributing to the question, this research aimed to investigate the technological production regarding the types of technologies and inventions available for therapeutic and diagnostic interventions in Chagas disease.

METHODS

In the present patent review, research used as a source for data collection the bases European Patent Office (EPO) and Cortellis Drug Discovery Intelligence (CDDI from Clarivate Analytics). As search descriptors the terms were used *Trypanosoma cruzi** AND *Chagas disease* (Figure 2). A total of 1.881 patents were identified for preliminary assessment from the databases and refined in the title and abstract fields (number of 208 patents) of which 138 were excluded due to being duplicates. After a careful check of the abstracts, n=31 patents being selected for critical analysis according to the study objetive (diagnostic and drugs). To establish whether the findings in these patents using in vivo and in vitro tests were supported by clinical trials using disease Chagas, a search was carried out on the website "clinictrails.gov" in December 2022, using the descriptor "disease chagas", obtaining 77 results from clinical trials. Clinical trials were included if Chagas disease was indicated in the title and the specified intervention included diagnostics and drugs.

Patent prospecting aims to understand commercial, scientific and technological trends through analysis of the geography and technology of the inventions produced (Queen's University Library, 2021). The design of this type of research is characterized by a systematic methodological strategy that can include different indicators such as temporal, technical, clinical trials, among several other dimensions (Bubela et al., 2013). Figure 2 illustrates the systematic search and screening strategy used in this review, which was based on the PRISMA stratement.



n = number of results retrieved.

Figure 2. Flowchart of patent search and screening.

These methods may be employed to create a systematic process of technological analysis, characteristics, development routes, and potential impacts on the future of research, development, and innovation (RD&I). For the present research, a specific time period was not established, thus making it possible to follow all the evolution of technological production on the subject. For the "drug" group, the following aspects were considered: geographical distribution of inventor countries; the time evolution of patent applications; the topic of patents size of molecules; the molecules under study and the stage of development of research related to these researches. The "diagnostic" group was considered the following aspects, distribution of patent applications; applicators and the characterization about the inventions.

Data were individually tabulated and organized into tables and graphs in Microsoft Excel (2019). From the data collected, the bibliometric treatment was carried out through the construction and analysis of networks based on the bibliography selected from the Software VOSview version 1.6.15 (van Eck and Waltman, 2010), whose emphasis is on the analysis and visualization of bibliographic data clusters using the distance-based approach.

Broadly speaking, the nodes of the bibliographic network are analyzed so that the distance between them approximately indicates their relationship, according to certain aggregation criteria, forming a map (Palludeto and Felipini, 2019). In this study, the criterion of co-occurrence (of relevant terms) was used, which seeks to identify elements common to the texts in the database and significant terms that are interconnected.

RESULTS AND DISCUSSION

Establishing new ways of intervening in public health problems is an urgent demand, especially in determining strategies to act on the ills that plague needy populations. NTDs lack pharmaceutical formulations that allow early care and diagnosis of affected populations. As identified, there are only two drugs currently in use to treat patients with Chagas disease, they are toxic and cannot be used for a long time to treat patients (Betina Moura, 2013; Thakare et al., 2021).

Understanding how the health technology market is acting on this issue is a crucial factor in proposing new intervention strategies for this health problem. Thus, resources under construction were observed to establish drug models and diagnostic methods proposed from the patents for these two segments. Initially, the candidates for drugs in development were observed according to the geographical distribution of the inventors, figure 3.

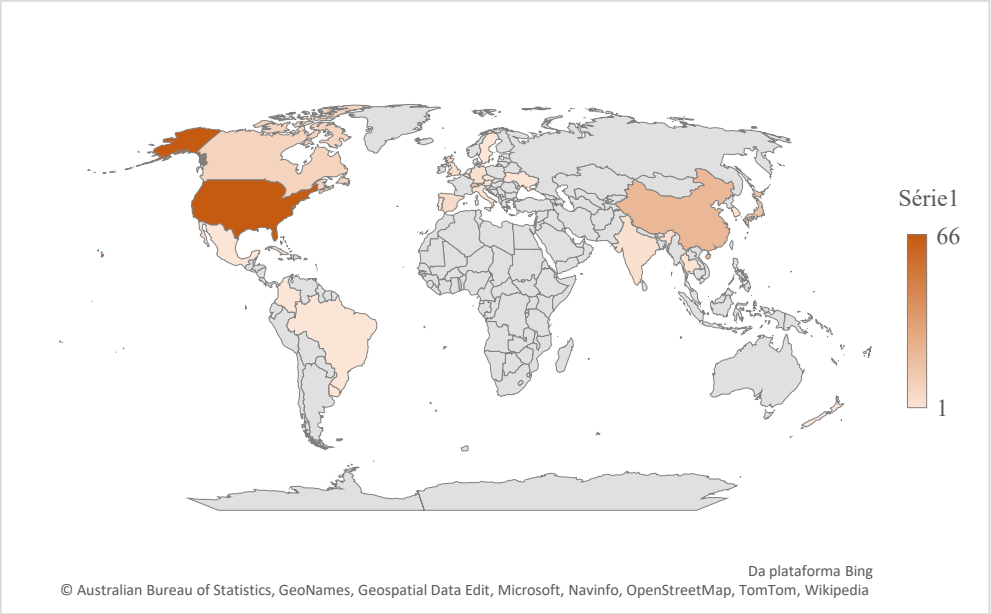


Figure 3. Distribution of patents referring to new molecules for the treatment of Chagas disease.

It is possible to verify that the main regions where technological production is accentuated refer to countries with stronger economies. Countries where the highest number of cases are observed, such as Latin America and Africa, have little or no patents for technological inventions to face the health problem. In view of the data, it is possible to verify that little is produced about technological resources of health care for the treatment of Chagas disease in the Americas, as already indicated by (Mendes et al., 2022).

Thus, some organizations (Tables 1 and 3) outside the endemic zone are interested in the development of drugs and/or diagnostics to treat the neglected infectious disease, Chagas disease, which, despite being an endemic disease in Latin America, and most cases of infection are reported in areas with low socioeconomic development (Caldas et al., 2019)

The analyzed patents showed that different research and development entities, like the industries, research institutes and universities have applied for patent rights for their inventions (Figure 4).

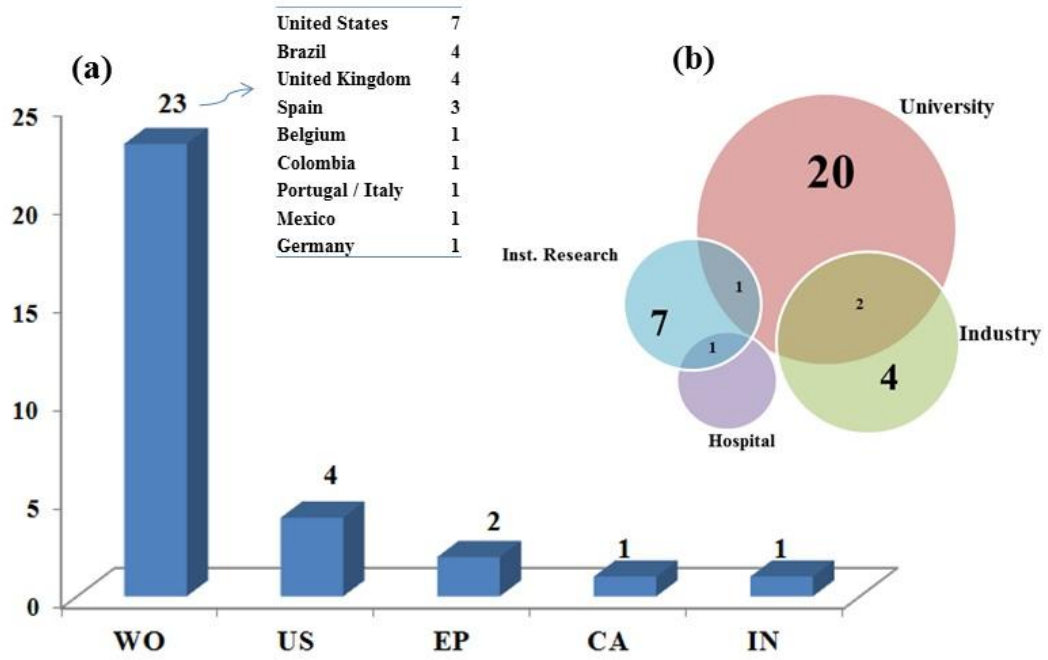


Figure 4. Number of patents by country and organizations. (a) Country according to the patent publication location – WO: World Intellectual Patent Organization; US: United State; EP: European Patent Office; CA: Canada; IN – India. (b) Patent applicants, for treatment of Chagas Disease a study of drugs and diagnostic methods.
Source: Elaborated by the author with CDDI (Clarivate Analytics) and EPO data.

Patents filed under the Patent Cooperation Treaty that assist applicants in seeking potential international patent protection for inventions are designated by the acronym WO (World Intellectual Property Organization), obtained the highest number n=23, however, the United States (US) despite having deposits of n=4 patents, there is also protection by WO, as well as the European Patent Office (EP). When checking the priority country that deposited via WO, the United States, Brazil, Belgium, Colombia, Spain, Portugal / Italy (they have joint ownership in the patented invention), Mexico and Germany are included (Figure 4 (a)).

The fact that the United States requests more patent applications may be related to its developed economy, high levels of investment in technology and innovation, and the culture of development in intellectual property and technological research (Alves et al., 2019; de Vasconcelos Cerqueira Braz et al., 2020). However, authors (Meymandi et al., 2017) state that the interest of the United States in investing in diagnostics and new drugs may be due to the number of people infected with *T. cruzi*, in a prevalence rate of Chagas disease of 1.24%, which is equivalent to more than 30,000. cases only in Los Angeles (Alpern et al., 2017).

The owners and/or inventors, who have the legitimacy to protect industrial property rights, are grouped into four main economic segments (industry, university, hospital and institute research). The universities lead the ranking with 23 patent deposits, for example,

Federal University of Minas Gerais (Brazil), Tulane University (United States), University of Texas System (United States), University of Dundee (United Kingdom), Federal University of Rio de Janeiro (Brazil), Universidad de Granada (Spain), University Mathura (India), University of Antwerp (Belgium), Federal University of Alagoas(Brazil), among others (Figure 4 (b)). It is worth emphasizing that industries and other research institutions have established cooperation with universities for product development.

Technological analysis of patents for Chagas disease

Pharmaceutical industries have little interest in the development of drugs for the treatment of Chagas disease, due to the high cost of investments and the lack of a potential market in developed countries. The inventors of the patent (LIDIA MOREIRA LIMA; et al., 2013) state that, in most cases, the development of drugs for the treatment of Chagas disease stems from others already approved for the treatment of other diseases, since they have already been submitted to clinical trials (Tables 1-3) (Alves, et. al.; 2012). Also, in the patent (TEIXEIRA, 2007a) the author, when describing the invention, discusses the new pharmaceutical compositions and associates to the already known antiparasitic agents, the inhibitors of the metabolic pathways of the host allowing the interaction of the kDNA of *T. cruzi* (Teixeira, 2006)

In patent number WO2014019044, (LIDIA MOREIRA LIMA; et al., 2013), proposes to synthesize and pharmacologically evaluate, in in vitro and in vivo models, new series of hydrazide-N-acylhydrazonic derivatives, designed as inhibitors of cysteinyl proteases, including cruzain or cruzipain and cathepsin, important cystenyl proteases found in epimastigotes, amastigotes and trypomastigoras of *T. cruzi* carry out the optimization step of the (S) prototype (S) identified (S) aiming at adapt its pharmacodynamic and pharmacokinetic properties, in order to ensure its passage to the drug candidate stage (Alves, et. al.; 2012).

In this same context, the products for which they are being patented, highlight the application of small molecules as drug candidates (Figure5) to be used for the treatment of the disease. In particular, small molecules with molecular weight >350-500 Da.

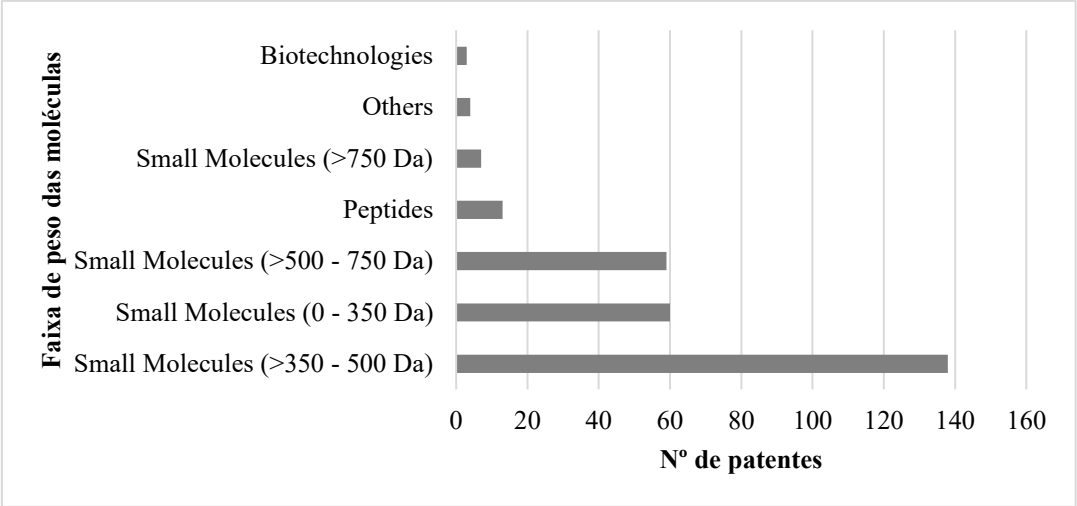


Figure 5. Molecular weight distribution of drug candidates in the treatment of Chagas disease.
Source: Elaborated by the author with CDDI data (Clarivate Analytics).

Investigating new compounds is extremely important for coping with the disease, mainly due to the lack of alternative intervention mechanisms that can be used in this confrontation. As indicated in the graph, several molecules have been investigated. Some of them are already in clinical studies, figure 6. The drug Odonacatib, one of the most studied molecules, belongs to the group of cysteine protease inhibitors, its target is the enzyme cruzipain, fundamental to the process of infection and virulence of *T. cruzi* (Castro and Soeiro, 2017; Ndao et al., 2014), becoming an important molecular target for the development of new drugs (Ferreira and Andricopulo, 2017).

This molecule has a patent application claiming its activity as an inhibitor of cysteine protease - cruzipain in a dose sufficient to prevent the parasite's infection process (CHAPLIN et al., 2013). Soon other patents that have inhibitory activity for Cathepsin K are indicated as showing activity against *T. cruzi* is the case das patente (Sedlmayr And Krekeler, 2015; Mão et al., 2016) identified during curation at CDDI.

The patents highlight the success of a multidrug treatment meaning parasitological cure and interruption of the evolutionary character of Chagas disease lesions and the pharmaceutical compositions claimed by the patents comprise at least one substance with antiparasitic activity selected from the group benznidazole, nifurtimox, ravuconazole (Wiesweg-Merkel and Just, 2020; Brand, et. al., 2017; Alves, et. al., 2012). Among the most used drugs in the treatment of Chagas disease, benznidazole and nifurtimox have significant activities in the acute phase of the disease, reaching rates of up to 80% of parasitological cure. Inventors of patent WO2018011739 report increased capture of antibodies produced by exposure to *T. cruzi* and high sensitivity and selectivity enabling detection of infection and effective monitoring of

treatment with benznidazole (Ferreira Marques and Tostes Gazzinelli, 2017a).

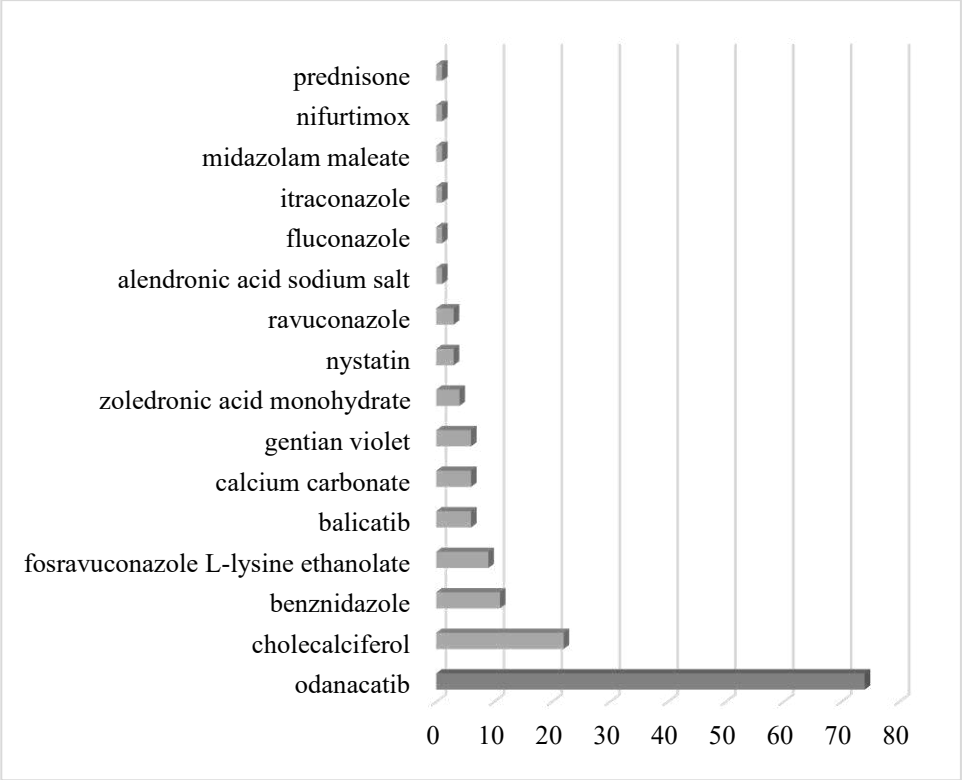


Figure 6. Number of studies by drug candidate for Chagas disease.
Source: Elaborated by the author with CDDI data (Clarivate Analytics).

However, most of these drugs are still in the early stages of research, as illustrated in figure 7. Demonstrating that there is still a long way to go for these new molecules to arrive in the health system. (Mendes et al., 2022) when analyzing the future perspectives for the development of drugs, it is evident the low number of molecules that reached the stages of clinical trials and less and a smaller number should be available on the market. The signal indicated by the researcher is reflected in the data collected during the research in the CDDI database and illustrated in figure 7.

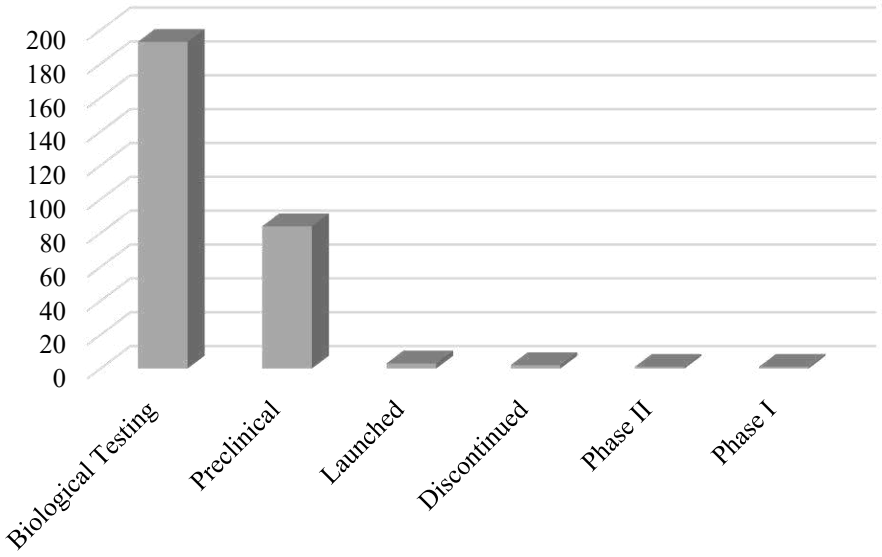


Figure 7. Number of drugs according to test phases.

Only two compounds are in phase 1 and 2 clinical trials respectively are the molecules NVP-LXE-408 and ravuconazole (Prop INN), figure 8. The compound LXE-408 known to have its activity evaluated against Leishmania was also evaluated against the Chagas disease parasite, T. brucei, with EC50 = 0.0039 mcM. The same already has characterization of the LXE-408 complex with the 20S subunit of the proteasome is recognized for the species of Leishmania tarentolae by electronic microscopy processes and deposited in the database Protein Data Bank (ID PBD: 6TCZ; 6TD5) (Nagle et al., 2020). Compound activity is centered on the ability to inhibit the proteolytic activity of the parasite proteasome (Iggart et al., 2020).

The compound ravuconazole (Prop INN), already known to present antifungal activity by inhibiting the activity of the sterol enzyme 14α-demethylase (CYP51) responsible for participating in the biosynthesis of sterols, necessary for the composition of eukaryotic cells (Riley et al., 2015). Some studies have evaluated and demonstrated its potential as a promising drug. (Betina Moura, 2013; G. Duschak, 2016), as well as the determination of the interaction profile between the drug and *T. cruzi* CYP51., PDB (ID: 3ZG3), (Hargrove et al., 2013). In vitro and in vivo tests indicate that the compound showed activity at minimum inhibitory concentrations (MIC) of 300 and 1 nM (Urbina et al., 2003). Curated data in the CDDI database indicating anti-protozoal activity for the compound, as in the patent application filed by (Ogawa and Yamaguchi, 2018).

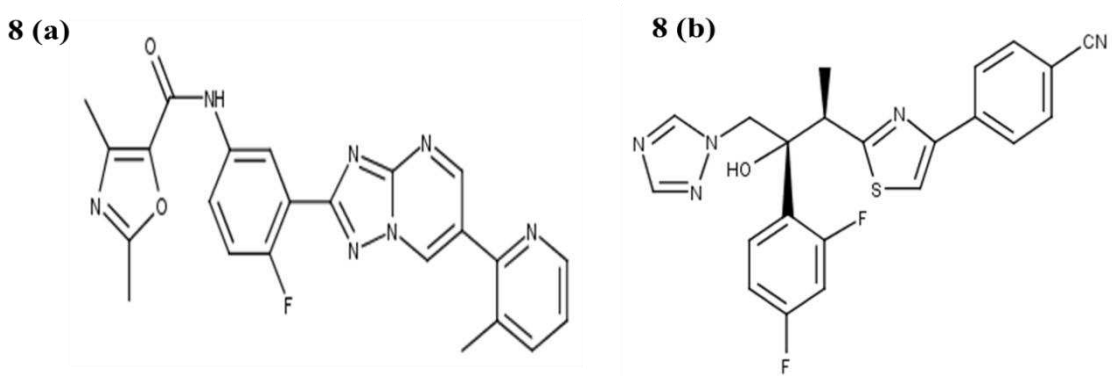


Figure 8. Two-dimensional structure of NVP-LXE-408 (**8(a)** PubChem CID: 118162630) and ravuconazole (Prop INN) (**8(b)** PubChem CID: 467825) compounds

Fonte: PubChen.

The progress observed in the area of drug development can be seen in Table 2, in which it is possible to verify that many of these compounds are still in the pre-clinical phase. Thus, it will still be some time before these products are available for application in health care practices.

Table 1.
Patents that describe diagnostic and drug to Chagas Disease.

Patent / Status	Applicant (Country)	Priority Year	Subject Matter	Condition	Compound Mechanism of Action	Technical field	Ref
IN202211055791 A1 - Invention patent application	GLA University Mathura (India)	2022	Carrier-mediated drug delivery Combination Products Nanotechnology	Trypanosomiasis, American (Chagas disease)	Angiogenesis Inhibitors Signal Transduction Modulators Adrenoceptor Antagonists Voltage-Gated Sodium Channel Blockers Calcium Channel Blockers Potassium Channel Blockers Vascular Endothelial Growth Factor Receptor 2 (VEGFR2; FLK1) Inhibitors Lanosterol 14alpha-Demethylase (CYP51) (Fungal) Inhibitors Oxysterol-Binding Protein 1 (OSBP; OSBP1) Ligands	Pharmaceutical composition comprising a dose of Itraconazole and a dose of Amiodarone and a method of administering this pharmaceutical composition in order to treat Chagas disease patients.	(Pathak, S and Bhardwaj, M. 2022)
WO2022187491 A1 - Invention patent application	Texas A&M University System University of Texas System (United States)	2021	Drug Substances	Trypanosomiasis	Cruzipain (*Trypanosoma cruzi*) Inhibitors	Cysteine protease inhibitors, in particular cruzain, cathepsin B and cathepsin L inhibitors.	(Meek et al., 2021)
WO2021076570 A2 - Published invention patent application without the search report.	Baylor College of Medicine (United States)	2019	Drug Substances Vaccines	Diagnostics Trypanosomiasis, American (Chagas disease)	DNA-Damaging Drugs	Immunogenic compositions comprising a recombinant protein.	(Han et al., 2020)
WO2020249500 A1 - Invention patent application	Bayer AG (Germany)	2019	Dosage Forms and Compositions	Trypanosomiasis, American (Chagas disease)	DNA-Damaging Drugs	Formulation comprising nifurtimox and to a process for production thereof, to the use thereof as a medication, and to the use thereof for prophylaxis and/or treatment of Chagas diseases	(Wiesweg-Merkel and Just, 2020)
WO2020256537 A1 - Invention patent application	Universidad Autonoma del Estado de Morelos Universidad Nacional Autonoma De Mexico (Mexico)	2019	Combination Products	Trypanosomiasis	Cell Membrane Disrupting Agents DNA-Damaging Drugs Ergosterol Ligands	A pharmaceutical composition for the treatment of trypanosomiasis or infections caused by *Trypanosoma cruzi*. The pharmaceutical composition includes: benznidazole; N-(L)-Histidinamide of amphotericin B.	(Espinoza Gutierrez et al., 2020)

US2020339619 A1 - Invention patent application	University of South Carolina (United States)	2019	Drug Substances	Infection, parasitic	Glucokinase (TcGlcK) (*Trypanosoma cruzi*) Inhibitors	Glucokinase (*Trypanosoma cruzi*; TcGlcK) inhibitors reported to be useful for the treatment of parasitic infections.	(D'Antonio, 2020)
WO2020188437 A1 - Invention patent application	Instituto de Biologia Molecular e Celular (IBMC) University of Modena and Reggio Emilia University of Porto (Portugal / Italy)	2019	Drug Substances	Trypanosomiasis, American (Chagas disease)	Pteridine Reductase 1 (PTR1) (Trypanosoma brucei) Inhibitors	Pteridine reductase 1 (PTR1) (Trypanosoma brucei) inhibitors reported to be useful for the treatment of American trypanosomiasis (Chagas disease). It displayed antiparasitic activity against amastigote form of *T. cruzi* strains (14.1 mcM).	(Costi et al., 2020)
WO2020016402 A1 - Invention patent application	Fundacio Privada Instituto de Salud Global Barcelona (ISGLOBAL) Hospital Clinic of Barcelona Institut d'Investigacio en Ciencies de la Salut Germans Trias i Pujol (Spain)	2018	Biomarkers	Trypanosomiasis, American (Chagas disease)	DNA-Damaging Drugs	A method for predicting the therapeutic response and/or prognosis in Chagas disease. The use of a protein expression product obtained from a biological sample of a chronic Chagas disease subject as potential biomarker in the context of said therapeutic response and prognosis.	(Fernandez Becerra et al., 2019)
WO2019150161 A1 - Invention patent application	Universidad de Antioquia (Colombia)	2018	Drug Substances	Trypanosomiasis, American (Chagas disease)	RAC Serine/Threonine-Protein (AKT; PKB)-Like Kinase (*Trypanosoma cruzi*) Inhibitors	Drugs include specific inhibitors of the AKT-like enzyme kinase of the *Trypanosoma cruzi* parasite.	(Marin Villa et al., 2018)
US2017157227 B2 – Granted patent (amended specification)	University of Texas System (United States)	2017	Drug Substances Vaccines	Trypanosomiasis, American (Chagas disease)	NA	Pharmaceutical compositions comprising an effective amount of peptide antigen, an adjuvant and a conjugate of said antigen, for the treatment of Chagas disease.A n exemplified product, MASPsyn-KLH, was trypanosoma vaccine consisting of a synthetic 20mer peptide derived from a mucin-associated surface protein (MASP) of *Trypanosoma cruzi*, linked to keyhole limpet hemocyanin (KLH).	(Maldonado Rosa A.; et al., n.d.)
WO2019106368 A1 - Invention patent application	University of Edinburgh (United Kingdom)	2017	Drug Substances	Infection, protozoal	ATP-Dependent 6-Phosphofructokinase (pfk) (Trypanosoma) Inhibitors	ATP-dependent 6-phosphofructokinase (pfk) inhibitors reported to be useful for the treatment of protozoal infections such as Trypanosomiasis.	(WalkinshaW et al., 2018)
WO2019076633 A1 - Invention patent application	Universiteit Ghent (UGhent) University of Antwerp (Belgium)	2017	Drug Substances	Diagnostics Trypanosomiasis	Viral Replication Inhibitors RNA-Directed RNA Polymerase (RdRp) (Viral) Inhibitors	Nucleoside analogs reported to be useful for the diagnosis and/or treatment of trypanosomiasis.	(Hulpia et al., 2018)

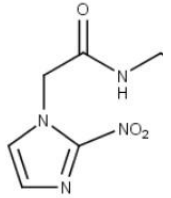
US2018280404 A1 - Invention patent application	University of South Carolina (United States)	2017	Drug Substances	Infection, parasitic	Glucokinase (TcGlcK) (*Trypanosoma cruzi*) Inhibitors	Glucokinase (*Trypanosoma cruzi*; TcGlcK) inhibitors reported to be useful for the treatment of parasitic infections. The compound inhibited TcGlcK activity (IC50 = 4.8 mcM). It displayed antiprotozoal activity against *T. cruzi* infective form (amastigotes and trypomastigotes) (Tulahuen strain) in murine embryo NIH-3T3 fibroblasts (EC50 = 2.983 mcM).	(D'Antonio, 2018)
WO2017195069 A1 - Invention patent application	Anacor Pharmaceuticals Inc (United States)	2016	Drug Substances	Trypanosomiasis	Cleavage and Polyadenylation Specificity Factor Subunit 3 (CPSF3) Inhibitors	Oxaborole ester compounds and compositions thereof which are useful to treat diseases associated with parasites, such as Chagas Disease	(Akama et al., 2017)
WO2018011739 A1 - Invention patent application	Universidade Federal de Minas Gerais (Brazil)	2016	Biomarkers Kits	Trypanosomiasis, American (Chagas disease)	NA	A method diagnostic and a kit for detecting anti-alpha-Gal antibodies by ELISA for the diagnosis of Chagas disease.	(Ferreira Marques and Tostes Gazzinelli, 2017)
WO 2017160849 A1 - Invention patent application	Baylor College of Medicine Tulane University (United States)	2016	Drug Substances Vaccines	Diagnostics Trypanosomiasis, American	Stimulation of the immune system for antibody production	Immunogenic compositions comprising a recombinant protein, claimed to be potentially useful for the diagnosis and treatment of *Trypanosoma cruzi* infection (Chagas disease).	(Asojo et al., 2017a)
WO2017178660 A1 - Invention patent application	PepTcell Ltd. (United Kingdom)	2016	Drug Substances Therapeutic Proteins Vaccines	Trypanosomiasis, American (Chagas disease)	NA	A Trypanosoma antigen may trigger an immune response that produce an α-Trypanosoma antibody capable of binding an epitope present on the surface of a Trypanosoma-infected cell.	(Pleguezuelos Mateo et al., 2017a)
WO2017025416 A1 - Invention patent application	GlaxoSmithKline Intellectual Property Ltd. University of Dundee (United Kingdom)	2015	Drug Substances	Trypanosomiasis, American (Chagas disease)	Proteasome Inhibitors	A compound or a salt thereof, processes for its preparation and its use in therapy, for example in the treatment of parasitic diseases such as Chagas disease. The compound with antitrypanosomal activity against *Trypanosoma cruzi* trypomastigotesm in rat H9c2 cardiomyocytes (pEC50 = 7.4, in intracellular assay) with reduced cytotoxicity against H9c2 cells (pEC50 < 4.3).	(Brand et al., 2016)
WO2016055607 A1 - Invention patent application	GlaxoSmithKline Intellectual Property Ltd. New York University (United Kingdom) (United States)	2014	Drug Substances	Trypanosomiasis, American (Chagas disease)	NA	Compound reported for the treatment of American trypanosomiasis (Chagas disease). The compound, 3-(benzo[d]thiazol-5-yl)-5-(methylamino)-l-(2,2,2-trifluoroethyl)-lH-pyrazole-4-carboxamide, salts thereof, in the treatment or prevention of Chagas disease.	(Alonso Padilla et al., 2015a)

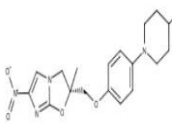
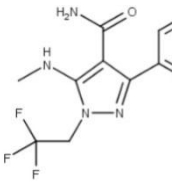
WO2009086398 A2 - Published invention patent application without the search report.	Abbott Laboratories (United States)	2007	Antibodies Drug Substances	Diagnostics Trypanosomiasis, American	Immunodiagnostic	Immunodiagnostic reagents comprising one or more antibodies specifically binding to diagnostically relevant region of *Trypanosoma cruzi* polypeptide such as FP3, Pep2, FP10 and FRA, for diagnosis of american trypanosomiasis (Chagas disease).	Brophy et al., 2008
WO2008051782 A1 - Invention patent application	Abbott Laboratories United States	2006	Biomarkers	Diagnostics Trypanosomiasis	Immunodiagnostic	The diagnosis of *trypanosoma cruzi* infection, methods of identifying and diagnosing *Trypanosoma cruzi* infection using a combination of four recombinant polypeptides.	(Shah et al., 2007)
WO2008022422 B1 - Granted patent	Universidade de Brasilia (UnB) (Brazil)	2006	Drug Substances	Diagnostics Trypanosomiasis	*T. cruzi* kDNA integration inhibition	The pharmaceutical compositions in the substance association having antiparasitic activity with inhibiting substances of signaling pathways that make possible the integration of kDNA of *T. cruzi* in the host genome.	(Teixeira, 2007a)

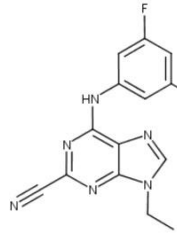
* Data Not Available – N.A. **Source:** Elaborated by the author with CDDI (Clarivate Analytics) and EPO data.

Table 2.

Possibilities for specific with drugs of Chagas Disease.

Patent	Drug Name	Phase	Administration Route	Product Category	Experimental Activity	Method	Parameter	Formulation	Chemical structure	Ref
WO 2021076570	Ro-7-1051 YC42NRJ1ZD benznidazole Abarax	Launched - 1971	Oral	DNA-Damaging Drugs	Antitrypanosomal Agents	Recombinant protein, potentially useful for the diagnosis and treatment of *T. cruzi* infection	MED	Dispersable tablets, 12.5 mg Tablets 12.5 and 100 mg		(Zhan et al., 2020)
US 2017157227	MASPsyn-KLH	Preclinical	Intraperitoneal	Peptides Peptide Vaccines Trypanosoma Vaccines	Antitrypanosomal Agents	Evaluation of the Humoral Immune Response (ELISA assay)	NA	20µg/mouse of MASPsyn alone and combined with 0.9% aluminum hydroxide	NA	(Maldona do Rosa A.; Serna Carylinda ; Almeida Irog C., 2017)
WO 2017178660	CH-47	Biological	Subcutaneous	Peptide Vaccines	Trypanosoma	Secretion of	NA	NA	NA	(Pleguez

		Testing		Polypeptides, from 10 AA to 40 AA Trypanosoma Vaccines Therapeutic Proteins	Antigenicity	cytokines like interferon gamma (IFN-gamma) and interleukin-4 (IL-4) (ELISA Assay)				uelos Mateo et al., 2017b)
WO 2017160849	Tc24-C4	Preclinical	Miscellaneous routes, for example, topical (i.e., transdermal) administration, mucosal administration (intranasal, vaginal, etc.) and/or inhalation	Polypeptides, from 41 AA Recombinant proteins Recombinant Vaccines Trypanosoma Vaccines	Antitrypanosomal Agents	Recombinant protein, potentially useful for the diagnosis and treatment of *T. cruzi* infection	NA	NA	NA	(Asojo et al., 2017b)
WO 2017072523	Delamanid	Launched - 2014	Oral or intravenous	Nitroimidazoles, Antiinfective Agents Prodrugs	Trypanosomiasis remission/reduction, IN VITRO	Amastigote assay	IC-50	Twice daily doses of 0.5 to 2 mg/kg		(Fairlamb et al., 2016)
WO 2016055607	Benzothiazole derivative {3-(benzo[d]thiazol-5-yl)-5-(methylamino)-1-(2,2,2-trifluoroethyl)-1H- pyrazole-4-carboxamide}	Biological Testing	Topical	NA	Trypanosomiasis remission/reduction, IN VITRO	Trypomastigote assay; Amastigote assay	IC-50	0.01-1% of the active compound		(Alonso Padilla et al., 2015b)
US 2015099795	Antisense oligonucleotide suppressing the expression of an inositol 1,4,5-trisphosphate receptor protein of *Trypanosoma* parasites	NA	1) in a form which is contained as it is in the therapeutic agent or 2) in a form which is incorporated in a downstream of an appropriate promoter sequence (antisense RNA expression vector)	Antisense Therapy Single stranded oligodeoxyribonucleotide (DNA)	Antitrypanosomal Agents	An antisense oligonucleotide used as a medicinal component to suppress the expression of the IP3R protein	NA	NA	NA	(Mikoshiba Katsuhiko; et al., 2014)
WO 2014019044	Hydrazide-N-acylhydrazone compounds	Preclinical	NA	NA	Antitrypanosomal and Antileishmanial Agents	Molecular hybridization between the	NA	NA	NA	(Lidia Moreira Lima; et

						LASSBio-1111 (leishmanicidal) prototype and the LASSBio-1064 (trypanocidal) prototype				al., 2013)
WO 2010059418	Substituted triazine and purine compounds	Preclinical	Oral, parenteral or topical	Cruzipain (Cruzain) Cruzipain precursor (Tc00.1047053507603.270)	Cathepsin B inhibition, IN VITRO	Cathepsin B, inhibition	IC-50	Daily dosages of from about 0.03 to 2.5 mg/kg per body weight		(Thomas et al., 2009)

* Data Not Available – N.A. **Source:** Elaborated by the author with CDDI (Clarivate Analytics) and EPO data

On a first view, it highlights the fact that only a small number of patents developed over the years fit our search criteria (10). Chagas Disease affects 6 to 7 million people all over the world (WHO, 2021), and Benznidazole, fisrt lauched in 1971, as seen in patent WO 2021076570, has been used as first-line drug treatment over the last 30 years (Teixeira, Silva et al. 1990) especially in several regions of Latin America (de Moura Ferraz, Alves et al. 2018), mostly due to fewer side effects and better tolerance (Bern, Montgomery et al. 2007).

Last decade patent deposits describes inventions whose focus ranges since drug assays activity against amastigote (WO 2017072523) and trypomastigote (WO 2016055607) parasite forms to peptide vaccines with antitrypanosomal agents activity (US 2017157227) and trypanosoma antigenicity activity (WO 2017178660).

In some clinical scenarios, CD diagnosis by serological methods is not applicable. Therefore, molecular tests can be used to detect the pathogen, but it requires well-equipped laboratories and technical knowledge, not always available in low-resources regions (Alonso-Padilla, Gallego et al. 2017). Molecular techniques are precise useful tools for CD diagnosis, even more when used at acute phase detection, due their high discriminative power (Hernández, Cucunubá et al. 2016).

Concerning to the information presented in Table 2, it's noted that biomolecular technologies were highly implemented as development methods, like gene suppression, molecular hybridization and protein recombination. The patents herein discussed tried to breach trough several scientific obstacles to produce better ways for CD treatment.

For example, patent WO 2010059418 focused on triazine and purine substitute compounds to treat and prevent mammalian protozoal disease, CD included, by Cathepsin B inhibition, an enzyme crucial for the parasite's life cycle evolution. Recombinant Tc24-C4, is an example of composition suggested in patent WO 2017160849 that works as a vaccine providing immunization, preventing or treating *T. cruzi* infection in mice. Patent WO 2014019044 proposes to use hydrazide-N-acylhydrazones molecularly hybridized compounds (*LASSBio-1111* and *LASSBio-1064*) in order to inhibit cysteine proteinases involved in infectious processes related to *T. cruzi* parasite.

In other hand, despite the well-established therapeutic method that uses RNA interference to silence disease related genes (Barker Jr, Metelev et al. 1996), this treatment has no effectiveness against *T. cruzi* once the dsRNA degradates in epimastigote and amastigote parasite forms (DaRocha, Otsu et al. 2004). In order to solve this problem, patent US 2015099795 suggested to develop an antisense oligonucleotide that suppresses the expression of an inositol 1,4,5-trisphosphate receptor protein of Trypanosoma parasites.

Patent and Clinical Trial for Chagas Disease

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There is a gap between patent time and clinical trials, authors (van de Burgwal et al., 2018; Wiegers et al., 2022) posed point out two factors, one of which is that the patent application corresponds to a previous step and/or during the research process and the execution of the clinical trial takes place when the bench test is proven. The second factor, the patent application process is relatively simple and less costly than running a clinical trial. This, therefore, may cause a gap between the number of patents filed and the number of clinical trials performed (Wiegers et al., 2022).

Benznidazole is the only drug approved by the FDA for the treatment of Chagas disease, however, this drug has low efficacy in the chronic phase of the infection and marked toxicity during treatment (Marques, et. al., 2018). In addition to this drug, the use of Nifurtimox is also mentioned. Both drugs introduced in the mid-1960s and 1970 (Wendel et al., 1992).

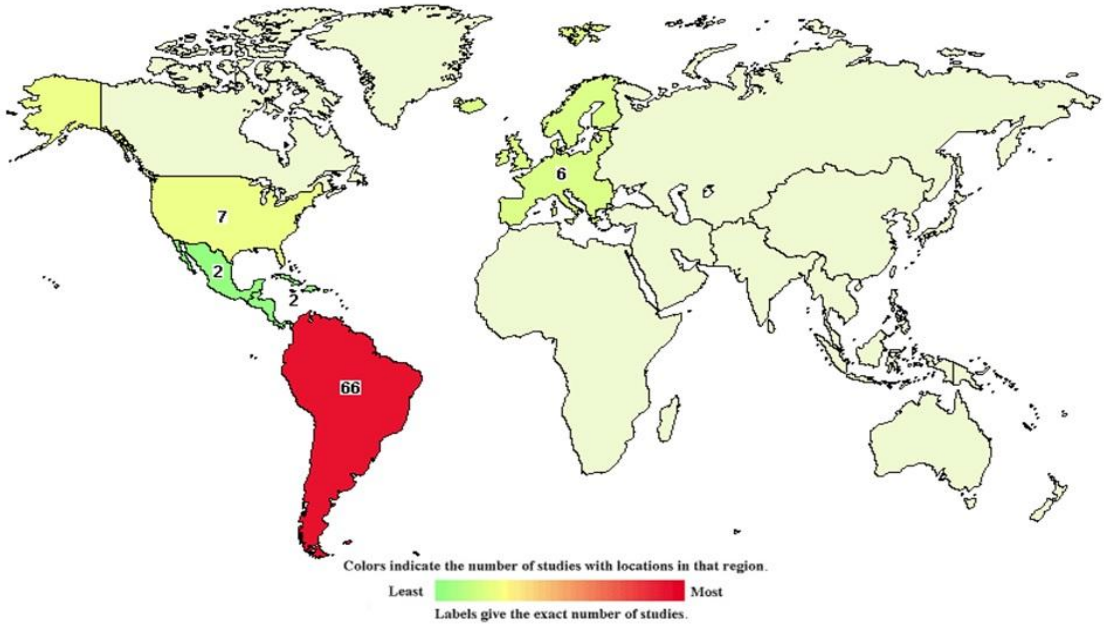


Figure 9 – Clinical Trials around the world for Chagas Disease.
Fonte: clinicaltrial.gov

It is noteworthy that, in the initial chronic phase of CD infection, two randomized clinical trials were carried out using Benznidazole (BNZ), controlled by placebo, where the efficiency of the drug was tested in regions of Brazil and Argentina with approximately 750 patients in ages 18 to 75 years, seroconversion was achieved in 58% of Brazilian patients at the end of three years and 52% of infected people in Argentina at the end of four years (Marin-Neto et al., 2008). It should be noted that for South America, 66 clinical studies are registered, according to the Clinical Trial (2023).

The mechanism of action of BNZ is still not fully understood, so far, it has been considered a prodrug due to its trypanocidal effect, which depends on enzymatic activation by trypanosomal type I nutrireductases, a group of enzymes expressed in protozoa. parasites (Losada Galván et al., 2021).

Both Benznidazole and Nifurtimox are administered when clinical symptoms begin to be observed, but the effectiveness of the drugs is reduced when comparing the use at the beginning of the infection, in relation to the use in an advanced infectious stage (Aldasoro et al., 2018). It is noteworthy that one of the obstacles to the rapid initiation of treatment is related to the silent progression of the pathology, preventing the clinical diagnosis in many cases (Müller Kratz et al., 2018).

In an initial clinical study with 73 patients infected with CD and treated with Benznidazole, the cure rate reached 88%. In addition, three types of doses were tested in 33 individuals with chronic infection: the first, with 7-10 mg/kg/day for 60 days; the second administration of 7-10 mg/kg/day for 30 days and the third 4-5 mg/kg/day for 30 days. There were no statistical differences between treatment effectiveness, with 90% of negative xenodiagnoses (Ciapponi et al., 2020).

The clinical pharmacological and toxicological profile of drugs used in the treatment of CD, potential concerns include teratogenicity, effects on male and female fertility, genotoxicity and carcinogenicity (problems made worse by infection) (Patterson and Wyllie, 2014).

It is known that in Chagas disease there may be aggravation of other clinical conditions, for example, cardiac function, mention is made of myocardial fibrosis that affects not only the sinoatrial node (NSA) and Purkinje fibers, but also the ganglia intracardiac nerves and the contractile myocardium (Noheria and Anderson, 2021).

In this sense, in a clinical study, in which 58 adult individuals aged between 18 and 70 years with CD were invited, cardiac functions were analyzed through tests such as: electrocardiogram, echocardiogram, chest X-ray and blood analysis. It was possible to verify that 43% of the individuals examined during the clinical study had some type of ventricular dysfunction, which demonstrates the need for constant monitoring in cardiac patients affected by CD (Macedo et al., 2015).

Despite constant advances, it is necessary to intensify phase I and II clinical studies, in search of new drugs and medications used for the treatment of CD, as fixed and adjusted doses of drugs such as Benznidazole and Nifurtimox can help in a safe and effective treatment (Caldas et al., 2019).

Technology applied to the diagnosis of Chagas disease

As already indicated initially, diagnostic strategies are required to combat parasitosis and provide prior assistance to the disease. In this sense, only 10 patents are applied for this purpose, table 3. As in the development of drugs, the production of diagnostic resources is scarce, making it difficult to monitor and prevent new cases. Fundamental action to fight the disease (World Health Organization, 2010).

The mapping of technology around the diagnosis demonstrates that the first patent registration was applied in 1990 with its growth in the first decade of the 2000s. When analyzing the set of patents on this subject, it is possible to observe that immunobiological tests prevail through the Enzyme Linked Immunono Sorbent Assay – ELISA. The emphasis of the tests is for the differential diagnosis of the stages of infection, mainly for the determination of the chronic stage of the disease as proposed in the patents (Reed and Goto, 2009; Teixeira, 2007b; Thomas Carazo et al., 2011).

The elements used for the diagnosis are varied, such as the use of the peptide proposed by (Thomas Carazo et al., 2011) sed to carry out the differential diagnosis. Propositions in this sense are diverse and allow differentiation during the diagnosis of the disease between the phases, chronic and acute, in the patient (de Pablos Torro et al., 2010; Travassos et al., 1998)he advances in methods also allow monitoring the immune response to the treatment performed in patients by monitoring targets such as (KMP11; HSP70; PFR2y/o; TGP63) (Lopez Lopez et al., 2010).

Another measurable parameter offered by the new diagnostic methods is the severity of the disease (Garg Nisha Jain, 2012; Teixeira, 2007b) or even the response to drugs such as benznidazole, one of the main alternatives in use for the treatment of the disease. (Ferreira Marques and Tostes Gazzinelli, 2017). The present method uses the action of conjugating several copies of carbohydrates of the Galα1-3Gal ϕ1-4GlcNAcR type to a solid particle of the Q-beta type, which leads to an increase in the number of captured antibodies after exposure to *T. cruzi*, thus how to monitor the effectiveness of treatment with benznidazole.

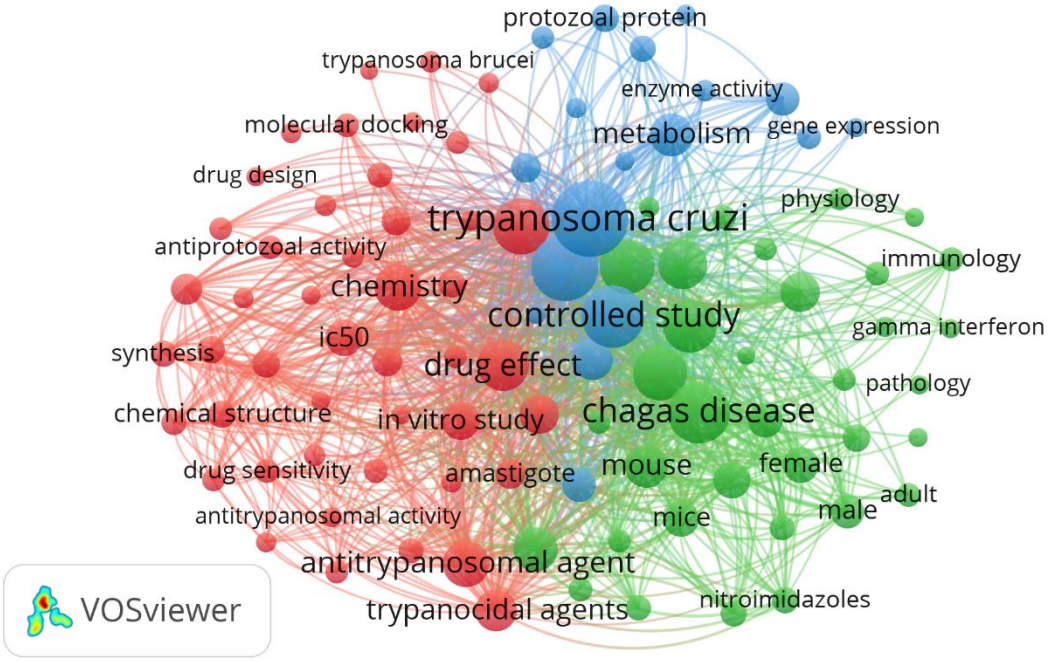
Tables 3. Patented technological products for diagnosis of *Trypanosoma cruzi*.

IDF Patent	Earliest priority	Title	Diagnosis Technique	Object of Diagnosis	Test Target	Ref.
WO 2018011739A1	2016	Virus Particle Linked To Carbohydrate Antigen For Differential Diagnosis Of Chagas Disease, Method, Kit, Vaccine And Use	Detection of anti-alpha-Gal antibodies by ELISA.	Diagnosis of *T. cruzi* and follow-up of treatment efficacy using benznidazole.	Antibodies to anti-alfa-Gal	(Ferreira Marques and Tostes Gazzinelli, 2017c)
US 2012316211A1	2011	Diagnostic Methods for Assessing Risk of Chagas Disease and Heart Failure	Technique can be used Western blot assays, ELISA assay, immunofluorescence, immunoprecipitation, radioimmunoassay or combinations thereof.	Assessment of the presence or severity of Chagas disease.	VIM, GSN, MYL2, MYH11, VCL e PLG.	(Garg Nisha Jain, 2012)
WO 2012007622A1	2010	Method for the Differential Diagnosis of Chagas Disease	Immunobiological Assay, ELISA	Diagnosis of Chagas Disease in the chronic phase; Differential determination of patients with chagasic and non-chagasic heart disease; Evaluation of response to treatment in patients with Chagas disease in the chronic phase.	Antibodies to *T. cruzi*	(Thomas Carazo et al., 2011)
WO 2010139826A1	2009	Use Of The Masp-52 Protein For The Diagnosis, Treatment And Prevention Of Chagas Disease	SDS PAGE and later West blot;	To determine the differential diagnosis for Chagas disease between distinct evolutionary forms of *T. cruzi*.	MASP-52	(de Pablos Torro et al., 2010)
WO 2010142829A1	2009	Method For Obtaining Useful Data For The Differential Diagnosis Of Chagas Disease, And For Evaluating The Response To The Treatment	Antibody detection: KMP11; HSP70; PFR2y; TGP63.	Evaluation of the response to the treatment of the referred disease.	KMP11; HSP70; PFR2y/o; TGP63	(Lopez Lopez et al., 2010)
WO 2009158729A2	2008	Compounds and Methods for Diagnosis and treatment af Chagas Disease	ELISA Enzyme Immunosorbent Assay	Diagnosis in the chronic phase.	Antibodies to *T. cruzi*	(Reed and Goto, 2009)
WO 2008022421A1	2006	Method And Kit For Diagnosis And Monitoring Of The Treatment Of Chronic Tripanossomiasis And Chagas Disease	Evaluation of the genomic DNA fragmentation profile from hybridization with specific kDNA integration markers.	Diagnose Chagas disease in the chronic phase and identify the severity of the disease.	kDNA	(Teixeira, 2007b)
CA 2309705A1	1998	Serological Diagnosis of Chagas' Disease	Chemiluminescent method (CL)-ELISA (Enzyme Linked Immunosorbent Assay).	Diagnosis in patients in the chronic and acute phase of Chagas disease.	Antibodies to *T. cruzi*	(Travassos et al., 1998)
EP 0486627A1	1990	*Trypanosoma Cruzi* Recombinant Antigens And Synthetic Peptides For Utilization Inthe Immunological Diagnosis Of Chagas Disease.	Immune detection.	Diagnose Chagas disease.	Antibodies to *T. cruzi*	(Oldenberg et al., 1991)
EP 0514509A1	1990	Composition And Its Preparation Process Using Antigen Conjugated To Enzymatic Activity For Immunological Diagnosis And Chagas' Disease Immunological Diagnosis Kits, For Individual And Epidemiological Application, Based On That Composition.	Diagnosis through ELISA assays or antigens immobilized on solid supports.	Diagnosis of Chagas disease infection.	Antibodies to *T. cruzi*	(Cerqueira de Almeida et al., 1991)

Source: Elaborated by the author with EPO data.

The specialized literature demonstrates the concentration of studies involving the development of drugs against *T. cruzi* centered on three segments, represented by clusters in red referring to chemical parameters and potential trypanocidal activity. The set of data referred to in Blue includes kinetic and metabolic parameters identified in the use of drug candidates and in Green comprises the series of studies involving in vivo biological assays. This data demonstrates the efforts being made to move forward on this topic. However, when we observe the prospected data on patents, it is possible to verify that few of these works managed to advance in the generation of a technological product and an even smaller amount is available in the pharmaceutical industry for use.

Figura 10. Mapping of relevant terms with the descriptors "*Trypanosoma cruzi* AND Drug"



As demonstrated by the data presented so far, it is possible to verify that research involving technologies applied to Chagas disease care is a fertile field with a high need for products that can diagnose and treat patients. Thus, prospecting for new compounds, whether of natural or synthetic origin, is a primordial action for the containment of new cases, safe treatment and a dignified life for people with the disease.

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3.2 CAPÍTULO 2: TRIPANOCIDAL ACTIVITY OF LECTIN FROM *Vatairea macrocarpa* ON THE EVOLUTIONARY TRIOMASTIGOTE FORM OF *Trypanosoma cruzi*, STRAIN Y

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Abstract: thousands of people all over the world, Chagas disease is at risk of becoming a worldwide epidemic. Facing this issue involves prospecting for new active principles. In this sense, the trypanocidal effect of the Vatairea macrocarpa lectin on the trypomastigote evolutionary form *Trypanosoma cruzi*, strain Y was evaluated. 31.2µg/ml, 62.5µg/ml, 125µg/ml, 250µg/ml, 500µg/ml and 1000µg/ml. Toxicity to host cells (LLC-MK2) was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. The protein between the GIPL and Gal-GalNAc-GlcNac glycans present in the cell membrane of *T. cruzi* and the lectin was estimated by molecular docking. In the in vitro tests, the lectin presented a CC50 index of 2744.00 ± 548.00; when inhibited by lactose, it was not possible to estimate this value for the collected concentrations. The values, LC50 of the lectin was 125.70 ± 22.70. The observed VML selectivity index (SI) was: 21.83, making it a candidate for further studies. Molecular anchoring showed a greater transmitter for GIPL glycans in relation to the Gal-GalNAc-GlcNac saccharide: GILP -2.6 kcal/mol and the (Gal-GalNAc-GlcNac): 3.5 kcal/mol. The data demonstrate that the saccharides present in the structure bind to the lectin domain of the protein. Trypanocidal action is promising and new research that helps to understand the action movements involved in the activity of the lectin, it is still valid to evaluate its action on other evolutionary forms of the parasite.
Keywords: Chagas disease, American trypanosomiasis, Lipophosphoglycans.

INTRODUCTION

American trypsomyosis or Chagas disease is a parasitosis caused by the protozoan *Trypanosoma cruzi*. (VELASCO; MURILLO, 2020). Marked by predominantly affecting populations plagued by situations of vulnerability, this parasitosis has a tendency to become a worldwide epidemic in the face of migratory movements. (VELASCO; MURILLO, 2020; ECHAVARRÍA, et al., 2021; HOCHBERG; MONTGOMERY, 2023). The absence of effective coping policies contributes to the advancement of the disease, as well as the scarcity of resources destined for the development of research on the subject (MILLS, 2020, EDWARDS, MONTGOMERY, 2021).

In this sense, there is an urgent need to develop new alternatives to intervene on this problem. In this regard, different strategies have been considered, such as diagnostic technologies (GYSIN, et al., 2022), drug repositioning (BELLERA, et al., 2020) the search for

new active principles (MAGALHÃES, et al., 2022), as well as interventions in ways of socially dealing with the topic with the population (LIU; CHEN; ZHOU, 2020).

A study involving the glycobiology of *T. cruzi* has indicated the presence of a variety of glycans associated with its plasma membrane ((LEDERKREMER; AGUSTI, 2009). Part of these glycans are associated with cell surface proteins, mucins, performing numerous activities from immunological escape to increased infectivity of the parasite (PEREIRA-CHIOCCOLA, et al., 2020).

By analyzing the composition of the glycans present in the evolutionary forms of *T. cruzi* epimastigotes, the following profile of monosaccharides galactose, glucosamine, mannose, sialic acid and traces of galactosamine and inositol was determined (SINGH, et al., 1994). Also according to the authors, the profile of glycans found in *T. cruzi* is significantly higher than that found in Leshimania.

Based on the profile of glycans and the role they play in the successful infection and dissemination of the parasite, carbohydrate-binding agents have been considered as a strategy for the development of new drugs (VALENTE, et al., 2019). However, the toxicity of lectins and peptides can be considered as an obstacle (VALENTE, et al., 2019). Given the availability of galactose recognition agents, it is possible to evaluate the effectiveness of galactose-binding lectins and explore the trisaccharide Galα(1,3)Galβ(1,4)GlcNAcα present in trypomastigotes (SCHOCKER, et al., 2016) .

Thus, lectins with affinity to galactose can be explored for their ability to exert trypanocidal activity. Vatairea macrocarpa lectin – VML, a galactose-binding lectin, has an estimated molecular mass between 122 and 130 kDa (CALVETE, et al. 1988), with its primary structure consisting of 240 amino acid residues (CAVADA, 1998). Composed of three chains α, β and γ with the respective masses 32-33, 22 and 13 kDa, its biological activity is described in different activities, which are the stimulation of the specific net efflux of H+ in Rhizobium tropici (Martínez et al., 2004), action Anti-biofilm action and Reduction in the adhesion capacity of bacteria Streptococcus mutans, S. sobrinus, S. sanguis, S. mitis, S. oralis (Texeira et al., 2005); Reduction in the growth of colonies of Staphylococcus aureus bacteria; S. epidermidis Pseudomonas aeruginosa, Candida albicans (Vasconcelos et al., 2014); antigenotoxic activity at concentrations lower than (0.5 and 2 mM) , Angiogenic activity and pro-inflammatory effect human lymphocytes and Gallus domesticus embryos (Veras et al., 2021), ability to increase blood pressure perfusion, renal vascular resistance , urinary flow and glomerular filtration rate in Wistar rats (Martins et al., 2005), antibiotic action in combination

with antibiotics such as gentamicin, norfloxacin and penicillin on strains of S*taphylococcus aureus* and *Escherichia coli*. (Santos et al., 2020). Given the vast existing application for the use of this protein, the aim of this work was to evaluate the trypanocidal effect of the *Vatairea macrocarpa* lectin - VML on resistant forms of *T. cruzi*, strain Y.

MATERIALS AND METHODS

Legal provisions

Vatairea macrocarpa seeds were collected from plants located in the city of Crato, in the state of Ceará, Brazil. A specimen of the plant was deposited and identified in the Herbarium of Andrade-Lima/Universidade Regional do Cariri and identified with the registration number: #15.114 and registered in the SISGEN (System for the Management of Genetic Heritage and Associated Traditional Knowledge, ID: A6D883E) .

Extraction and Purification

The purification process was carried out according to the Calvete protocol (1998). For this, a guar gum gel column (2 X 10 cm) was used. The VML fraction was lyophilized and verified its purity by SDS-PAGE (LAEMMLI, 1970).

Vatairea macrocarpa lectin (VML) was purified following the protocol adapted from Santos et al., (2020). Vatairea macrocarpa seeds were peeled and ground in an electric grinder until obtaining a fine powder. The flour resulting from this process underwent a delipidation process to remove fatty acids using the solvent, hexane. Soluble proteins were extracted in 0.15 M NaCl (1:10 w/v) under constant stirring at room temperature for 4 h. After centrifugation, the supernatant was filtered through a paper filter (Whatman™).

Affinity chromatography and dialysis

The clarified extract was used for affinity chromatography on a Guar Gum matrix (2 × 10 cm), previously equilibrated with 0.15 M NaCl. After washing the proteins that were not retained, the bound protein fraction was eluted with 0.1 M galactose. Fractions of 1.5 mL were collected at a flow rate of 2 mL/min, and all fractions were monitored by a spectrophotometer at a wavelength of 280 nm. The fraction containing the VML lectin was constantly dialyzed against distilled water and the resulting material was frozen and lyophilized.

Electrophoresis on SDS-PAGE

The electrophoretic profile of the protein was determined by electrophoresis in a polyacrylamide gel in the presence of sodium dodecyl sulfate (SDS-PAGE) as described by Laemmli (1970). The separation gel (12.5%) consisted of 2.65 mL of distilled water; 750 µL of 3 M Tris; 2.5 ml acrylamide/bisacrylamide; 60 µL of SDS; 30 µL of ammonium persulfate (100 $mg.mL^{-1}$) and 10 µL of TEMED. For the stacking gel (4%) 2.1 ml of water were used; 380 µl of 1 M Tris; 500 µL of acrylamide/bisacrylamide; 30 µL of SDS; 30 µL of ammonium persulfate and 3 µL of TEMED.

The following proteins were used as molecular mass markers: phosphorylase B, 97 kDa; bovine serum albumin, 66 kDa; glutamic dehydrogenase, 55 kDa; ovalbumin, 45 kDa; glyceraldehyde-3-phosphate dehydrogenase, 36 kDa; carbonic anhydrase, 29 kDa; trypsinogen, 24 kDa; trypsin inhibitor, 20 kDa and α-lactalbumin, 14.2 kDa. The crude extract was added to one of the wells and the sample from peak II of the affinity chromatography was added to the other. After electrophoresis, SDS-PAGE gels were stained with Coomassie® Brilliant Blue (R-350) and then destained with warm distilled water.

Evaluation of cytotoxicity in host cells

The determination of the cytotoxicity of the VML lectin was evaluated in LLC-MK2 cells in order to identify the selectivity of the VML lectin by *T. cruzi* in distinction from host cells. The LLC-MK2 cell line corresponds to epithelial cells from monkey (Macaca mulata) renal tubules, which were obtained for the present study at the Rio de Janeiro Cell Bank (BCRJ) and cultivated in DMEM medium (Dulbecco's Modified Eagle Medium) in pH 7.4 and supplemented with 10% Fetal Bovine Serum (SBF) and antibiotics (penicillin - 200 IU.mL-1 and streptomycin - 130 mg.mL-1).

The cultivation was carried out in sterile plastic bottles with dimensions of (75 cm2) in a CO2 study at a temperature of 37.0 ± 0.3° C and 5% of CO2. Cultivation continued until confluence was reached. Then, the medium was removed and the cells were washed in 5mL of sterile PBS with 1 mL of Trypsin/EDTA solution (0.25%/0.04%) and incubated for a period of 5 to 10 minutes at 37° C, and subsequently inactivated in 2mL of DMEM medium 10% FBS.

Then aliquots were transferred to new containers containing culture medium for the maintenance of new cultures. Cell preparation for freezing was performed after washing and cell displacement, these were centrifuged at 4000 Rotation Per Minute (rpm) for a period of 5

minutes, the medium was discarded and the pallet was resuspended in a freezing solution containing (95% sterile SBF and 5% DMSO) and then frozen in liquid nitrogen.

Cytotoxicity assay

The test was carried out using the MTT reduction method (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) initially described by (MOSMANN, 1983). The assay consists of the process of endocytosis of the MTT salt (yellowish salt) which is subsequently reduced by mitochondrial enzymes into an insoluble purple salt, the Formazan salt, allowing the assessment of the state of the cell's respiratory chain (RACUSEN, et al. al., 1999). The amount of Formazan produced is determined by reading in a spectrophotometer at 570nm and is directly proportional to the number of viable cells.

The confluent culture was washed in trypsinized solutions as done above. Transferred to falcon tubes and centrifuged at 4000 rpm for a period of 5 minutes, at the end, the supernatant was discarded. Then the pallet is resuspended in 1 mL of DMEM medium. An aliquot was taken and diluted in a trypan solution (0.4% m/v in PBS) in the following proportions 1:10 and 1:100. The second was then taken for the determination of cell density in a Neubauer chamber.

Determination of cell density

To determine the cell density, the following equation was applied

Equation 1: calculation for determination of cell density.

$$\text{Cell density} = \frac{Conta\text{Cell count}}{4}\ X\ 10^4$$

The cell density fit was set to 105 cells. mL-1 and then transferred to sterile 96-well plates of 200 µl/well volume. The plates were incubated overnight in a CO2 oven, the wells were treated with the VLM lectin at concentrations of 15.5 µg/mL, 31.2 µg/mL, 62.5 µg/mL, 125 µg/mL, 250 µg/mL, 500 µg/ml and 1000 µg/ml. For the negative control, untreated groups and cultures treated only with 0.5% DMSO were used.

Determination of the percentage of cell viability (%)

The determination of the percentage of cell viability was established through the equation

Equation 2: calculation of cell viability by the MTT reduction assay

$$\text{Cell viability} = \frac{\text{Abs T} - \text{Abs BC}}{\text{Abs CT} - \text{Abs BC}} * \text{X } 100$$

Where: Abs T – Test group absorbance;

Abs BC – Blank absorbance;

Abs CT - Absorbance of the control group.

The data allowed estimating the values of minimum concentrations to reduce cell viability and coefficient (CC50) by 50% using the non-linear regression method.

Obtaining trypomastigote forms of *T. cruzi* strain Y

Obtaining the trypomastigotes evolutionary forms of *T. cruzi* were acquired following the protocol by LIMA et al. (2016). Initially, the LLC-MK2 cell culture was grown in 25 cm2 bottles at a concentration of 1 x 10 5 cells. mL-1 in DMEM medium containing 10% FBS. Cultivation took place for 48 hours in a CO2 oven, the medium was replaced by DMEM 2% SBF without antibiotics and the cells infected with trypomastigotes at the rate of 20 parasites per cell.

After 72 hours, the medium was replaced, followed by the supernatant centrifugation process at 2800 rpm for 7 minutes, in order to obtain the trypomastigotes forms. This action is carried out up to the 6th day after infection. The cell density determination process was carried out in a Neubauer chamber. The parasites obtained were used in the tests to determine the effect of the lectin on the respective form of the parasite.

Evaluation of the trypanocidal effect in trypomastigote forms of *T. cruzi* strain Y

The assay for determining the activity of the VML lectin was carried out in a 96-well plate with a volume of 200µL, in which the lectin was incubated at the following concentrations (15.5 µg/mL, 31.2µg/mL, 62.5µg/mL , 125µg/mL, 250 µg/mL, 500 µg/mL and 1000 µg/mL), in DMEM 10% FBS medium and parasites at a concentration of 106 trypomastigotes/mL. Untreated parasites were used as a negative control and 0.5% DMSO was used as vehicle group.

The plate was incubated in a CO2 oven for 24 hours and later aliquots were removed to determine the number of parasites by observation and counting in a Neubauer chamber. For this purpose, the percentage of cell viability was established using the following equation:

Equation 3: Viability of parasites by Neubauer Chamber count

$$\text{Cell viability} = \frac{T}{CT} X\ 100\%$$

On what:

T - Count of viable parasites in the experimental group;
CT - Mean viable parasite count of the negative control group.

The lethal concentration capable of reducing the number of parasites by 50% (LC50) was determined by non-linear regression. The selectivity index (SI) of the action of the VML lectin on host cells and the trypomastigotes forms was also recorded according to the equation.

Equation 4 - Calculation of the Selectivity Index.

$$\text{IS} = \frac{CC_{50}}{LC_{50}}$$

On what:

CC50 - Concentration capable of reducing the viability of host cells by 50%. LC50 - Lethal concentration for 50% of the parasites.

Statistical analysis

The experiments were performed in triplicate, with n = 3. The results are presented as mean standard error (SEM). For statistical analysis, one-way or two-way ANOVA was performed, with the application of the Dunnet and p. Graphs were constructed using GraphPad prism 7 software (https://www.graphpad.com/scientific-software/prism/).

In silico analysis of interactions between VML lectin and *T. cruzi* lipophosphoglycans

Obtaining the binders

The PubChem database was consulted to obtain the three-dimensional structure of the disaccharide Lactose, (beta-Lactose), under the code: - PubChem CID 6134. three-dimensional structure**.**

Modeling of ligands

The ChemSketch software was used to model fragments of the Lipopolysaccharide structures, the Glycoinositolphospholipids (GILP). from *T. cruzi*. The molecule was designed from the experimental data obtained by Ferguson (1997). The Gal-GalNAc-GlcNac saccharide

(SCHOCKER, et al., 2015) was modeled and optimized in the program. available on the platform and model automatically generated by the ChemSketch program which generated the 3D images of the molecule.

Optimization of binders

Molecules were previously prepared for virtual screening through the Avogadro program (HANWELL, et al. 2012) (https://avogadro.cc/), considering the protonation state under pH 7.4 conditions, optimization of the geometry of molecules and subsequently brought to an energy minimum.

Molecular docking

Molecular docking calculations were performed using the AutoDock Vina program that uses the algorithm genetic. The strategy used was site-directed docking. The protein was treated as a rigid body and the binder was considered as a flexible body. The Gridbox was built with the following center box dimensions and the following dimensions were established: center center_x = -21,952, y = -19,851, center_z = -29,065 and size_x = 24, y = 22, size_z = 18. The number of evaluations was considered seed = 2009, num_modes = 100 exhaustiveness = 8. For the selection of the best model, the affinity energy values indicated by the program were considered. Afterwards, the best poses were analyzed using the PyMOL viewer and the figures used in this document were constructed. Interaction analyzes were performed using the PyMol software, considering residues located at a distance of up to 3Å.

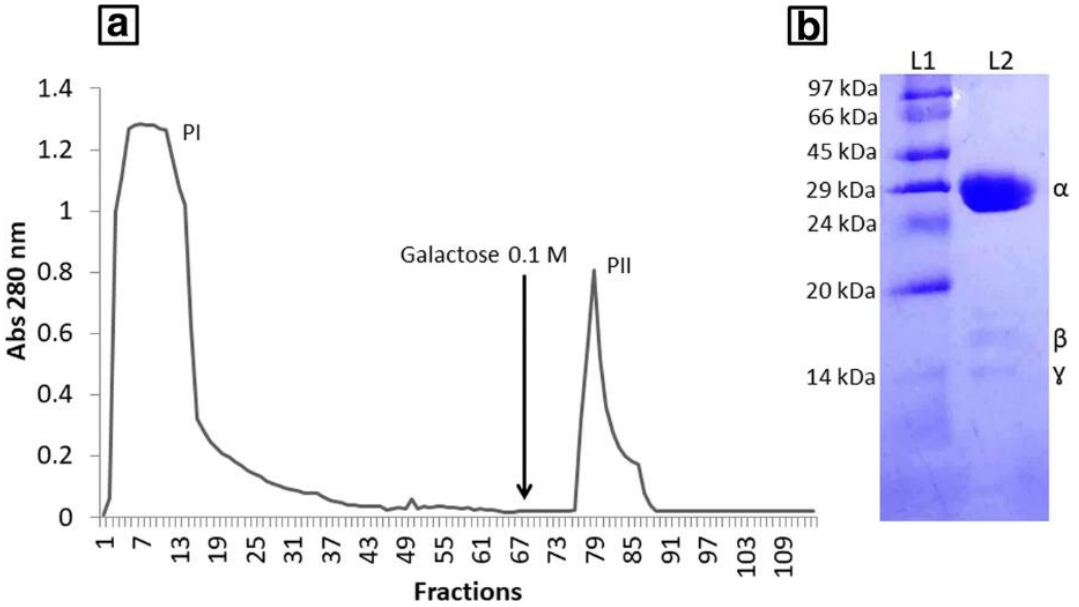
RESULTS AND DISCUSSION

VML lectin purification

The VML was purified by affinity chromatography on a Guar Gum column. The chromatogram (Graph 1) shows two peaks: PI and PII, where PI refers to proteins not bound to the column, that is, proteins of no interest, and PII refers to proteins retained after elution with galactose 0.1M, called proteins of interest.

The purity of this protein was confirmed by means of SDS-PAGE gel electrophoresis (Figure b). In the SDS-PAGE, the PII presented three bands, as shown in L2 of figure b, where the first band refers to the α chain with a mass of 29kda, the second band is the β chain with a mass of

18kda and finally the third band is the γ chain with a mass of 15kda. Thus confirming the purity of the VML lectin**.**

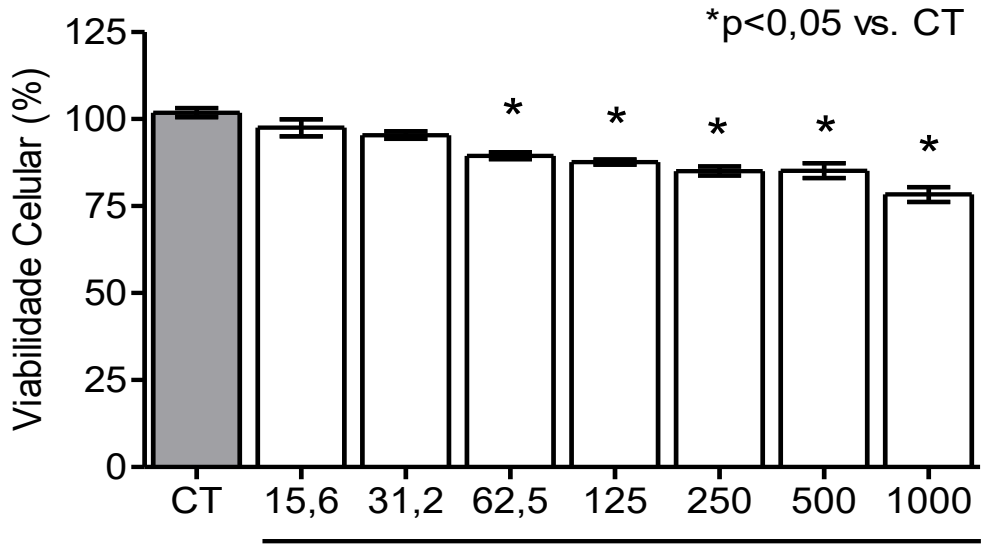


Graphic 1 – VML lectin purification comatogram by affinity chromatography and SDS-PAGE electrophoresis. Purification of VML by affinity chromatography. a - Chromatogram of the crude extract of Vaitarea macrocarpa on a Guar Gum affinity column. b - SDS-PAGE. L1: molecular mass markers (phosphorylase b, 97 kDa; bovine serum albumin, 66 kDa; ovalbumin, 45 kDa; carbonic anhydrase, 29 kDa; trypsinogen, bovine pancreas, 24 kDa; trypsin inhibitor, 20 kDa; and α lactalbumin, 14 Kda); L2: PII.

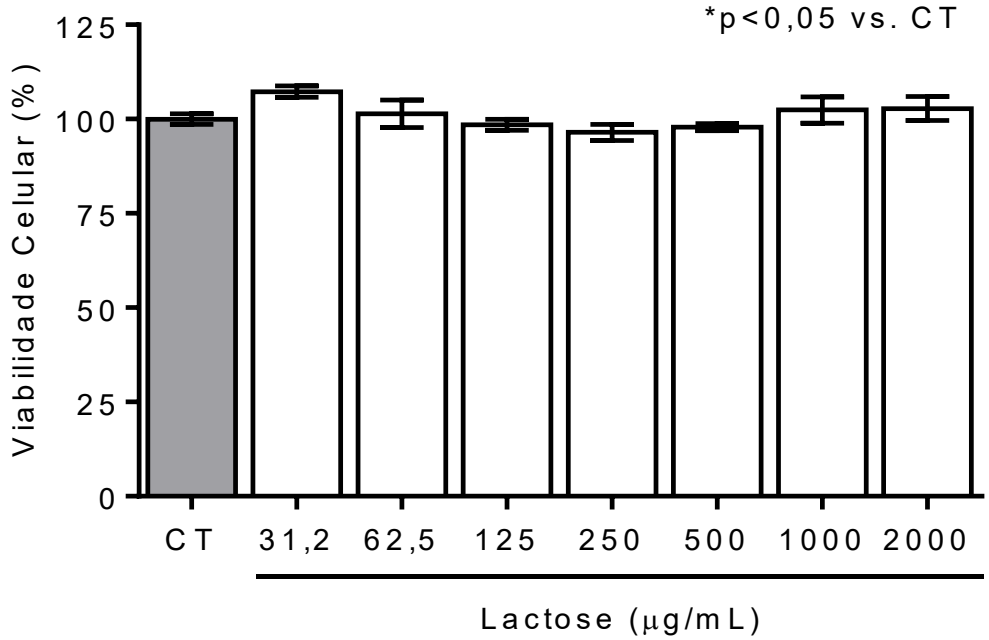
Host cell viability assay (LLC-MK2)

To evaluate the cytotoxic activity in Rhesus Monkey Renal Epithelial Cells (LLC-MK2 Line), the MTT assay (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazoline bromide) was performed for verify the production of metabolic damage related to exposure of cell culture to Vatairea macrocarpa lectin (VML). As indicated in table 1.

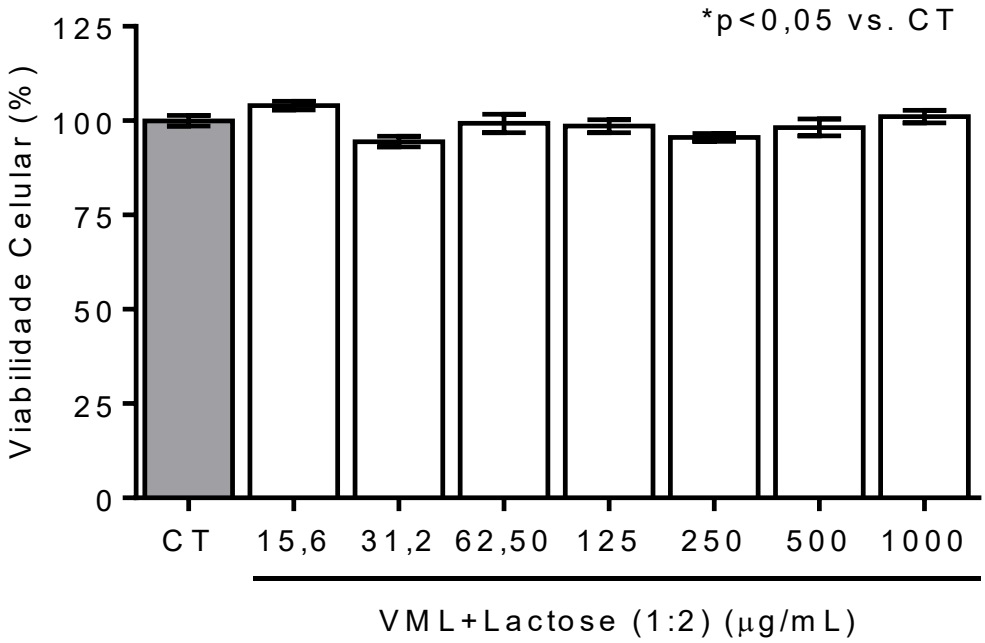
For evaluation of cell viability three conditions were tried. The first one, the viability assay of cells exposed to VML lectin Graph 2. Exposure of cells only to lactose (Graph 3) and exposure of cells to VML lectin inhibited by lactose. This action was proposed to verify whether the cytotoxicity of the lectin was related to its CKD or even if the disaccharide lactose would contribute to this effect.



286
287 **Graph 2** - CC50 – Estimation of the concentration capable of reducing the viability of LLC-
288 MK2 cells by 50%. (Lectin only).
289



290
291 **Graph 3** – CC50 – Estimation of the concentration capable of reducing the viability of LLC-
292 MK2 cells by 50%.
293



294
295 **Graph 4** - CC50 – Estimation of the concentration capable of reducing the viability of LLC-
296 MK2 cells by 50%. (Lectin + Lactose)

297 The data demonstrate that the VML presented low toxicity for the cell culture, enabling
298 the continuation of the tests. The same, there was already a description for the absence of
299 toxicity in other cell cultures, or even in an in vivo test (GONÇALVES et al., 2013), however,
300 there is a report of cytotoxicity in lymphocyte culture as reported by (VÉRAS et al. , 2022).
301 The determination of cytotoxicity parameters is crucial to evaluate the potential application of
302 the molecule in living organisms (LI; ZHOU; XU, 2015). When evaluating the cytotoxic
303 activity of the disaccharide lactose, it did not show cytotoxic activity in any of the tested
304 concentrations.

305 However, when the cytotoxicity of the lectin with its lactose-inhibited DRC was verified,
306 it showed a decrease in the degree of toxicity of the lectin in the culture of LLC-MK2 cells,
307 demonstrating that its toxic effect would be linked to the lectin domain of the protein. The
308 evaluation of the toxicity effect on other lectins, such as ConA, demonstrated a similar event in
309 an in vivo test (SANTOS, 2022).

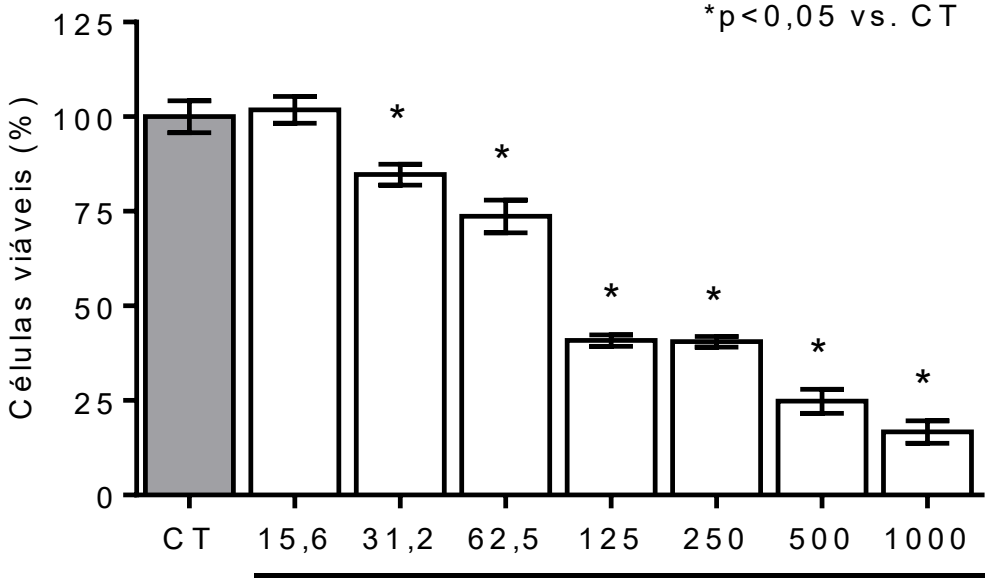
310 Regarding the ability to promote trypanocidal activity on the trypomastigote
311 evolutionary form, strain Y, of *T. cruzi*, the VML lectin showed Lc50 at a concentration of 40.85
312 at a concentration of 125 µg/mL as shown in table 1 and graph 5. It is known initially that some
313 lectins are capable of agglutinating parasites in this evolutionary stage as described for the lectin

314 from Cratylia mollis Cramoll 1,4 which was able to agglutinate the two evolutionary stages and
315 cause apoptosis in epimastigotes forms (FERNANDES et al. 2010).

316

Concentration	Viable cells (%)
CT	100,00 ± 4,25
15,6	101,80 ± 3,52
31,2	84,71 ± 2,74*
62,5	73,67 ± 4,34*
125	40,85 ± 1,50*
250	40,53 ± 1,43*
500	24,83 ± 3,18*
1000	16,70 ± 2,95*
LC_{50}	125,70 ± 22,70

317 **Table 1**. Trypanocidal effect on *T. cruzi* trypomastigotes. LC50 – Estimation of the
318 concentration capable of reducing the viability of LLC-MK2 cells by 50%.
319



320
321 **Graph 5** – Trypanocidal effect of the VML lectin on *T. cruzi* trypomastigotes.

322

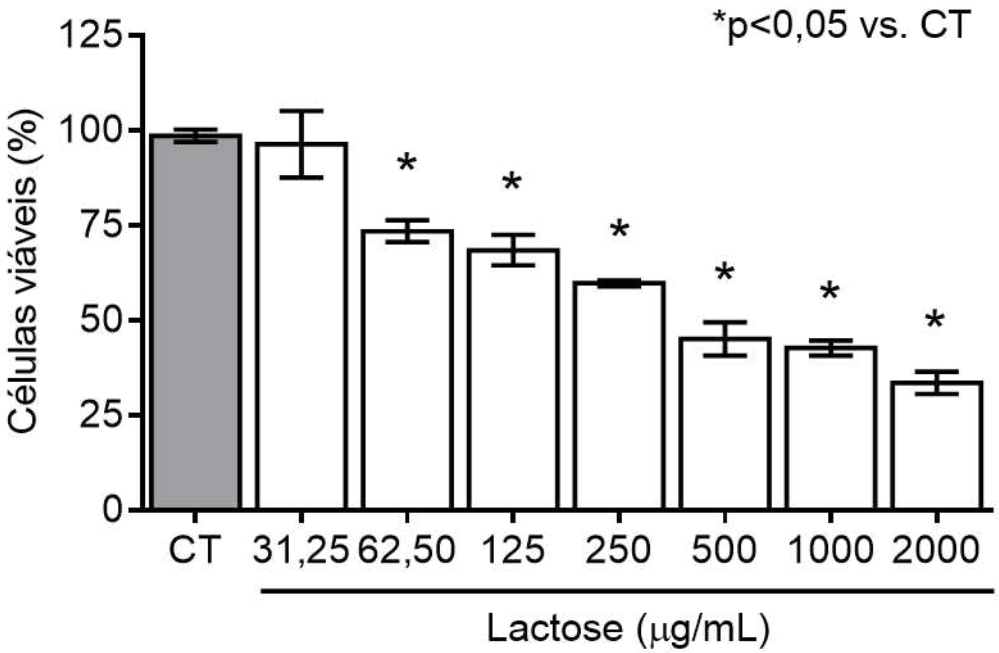
323 Studies involving the activity of plant-derived lectins in *T. cruzi* cell cultures
324 demonstrate that they can reduce the process of association between host cells and
325 trypomastigote evolutionary forms, in the case of Concavalin A (Stiles; Kierszenbaum, 1986;
326 Zenian; Kierszenbaum, 1982). When observing the way in which the lectins act on the infection
327 process, the role played by the Carbohydrate Recognition Domains (DRC) of the Ricinus
328 communis lectin was observed, demonstrating that the DRC are responsible for interacting with
329 D-galactose residues present on the surface of parasite membranes. D-galactose residues exert

a very important action, as they are responsible for interacting and mediating the recognition and actions for the infection of cardiac muscle cells of the host (BARBOSA; MEIRELLES, 1993).

In research involving different strains of *T. cruzi*, lectins have helped in determining the composition of the types of sugars present in the cell membrane of the parasite (ARAUJO et al., 1980). Knowing the glycobiology of *T. cruzi* has proven to be fundamental, as these carbohydrates are involved in different processes related to adhesion, recognition and evasion of the immune system (ALVES; COLLI, 2007). Other studies involving the Arachis hypogaea lectin (peanut agglutinin) demonstrated the potential use of these proteins as a tool to identify subpopulations of *T. cruzi* from the forms extracted from the intestine of the vector, based on the composition of the sugars present among the different types strains (ARAÚJO; MELLO; JANSEN, 2002).

However, research describing the trypanocidal activity of lectins is scarce in the literature. Thus, the research carried out contributes to expanding the biotechnological potential of the group of proteins, given the various descriptions of biological activities such as the action on kidney cells (MONTEIRO et al., 2010) already identified for the lectin. Considering health technologies, the VML lectin can be used in the evaluation of in vivo activity, such action is fundamental so that new molecules can be incorporated in more studies related to health care, especially in neglected tropical diseases such as Chagas disease.

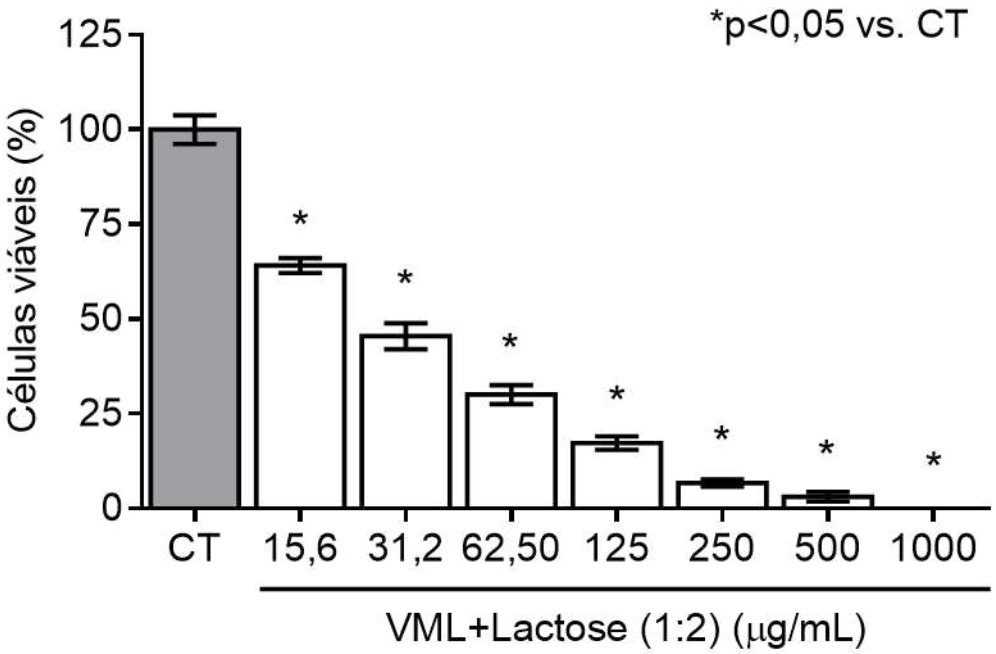
When evaluating the effect of lactose on the trypomastigote evolutionary form, it promoted the death of the parasites, graph 6. It is notorious that from the concentration of 62.50 µg/mL, the carbohydrate starts to promote the death of the parasites by the effect of osmolarity. Thus, it was not possible to determine whether the trypanocidal activity of the VML lectin is directly related to its DRC. It is likely that the action of lactose is related to the osmotic effect on cells, considering the concentration used in the experiments.



359

360 **Graph 6 -** Action of lactose on the evolutionary form of *Trypanosoma cruzi*, strain Y

361 A more pronounced effect on trypomastigotes was observed when VML lectin was
362 combined with lactose graph 7. The effect can be understood by the joint action of the lectin
363 that had already demonstrated action on this evolutionary stage and the concentration needed
364 to inhibit it, which was considered lethal in the previous test. Thus, the increase in trypanocidal
365 action was accentuated in the experiment. Other strategies can be thought of to verify the
366 relationship between CKD and the trypanocidal effect of the lectin. For this, protein
367 demetallization can be performed, given that the Mn2+ and Ca2+ ions are necessary for the
368 architecture of the CKD in the protein (LORIS, et al., 1998; BOUCKAERT et al., 2000). The
369 removal of these metallic ions compromises the recognition of carbohydrates in a specific way
370 by the protein (LORIS et al., 2004).



371

372 **Graph 7 -** Trypanocidal action of the VML lectin combined with lactose

373 For this purpose, the Selectivity Index (SI) was determined from the ratio between the
374 Concentration of the compound that reduced 50% of the viability of the macrophages (CC50)
375 and the Concentration of the compound that inhibited 50% of the parasite growth. IS values >
376 20 indicate that the compound is more toxic to the parasite than to host cells (DON; IOSET,
377 2014). In view of the tests carried out, values of 21.83 (> 7.96) were verified for the activity of
378 the VML lectin, as shown in Tabela 2.

379

	Estimated value
CC_{50} (VML)	2744,00 ± 548,00 (> 1000)
CC_{50} (VML + Lactose)	Não foi possível estimar (> 1000)
LC_{50} (VML)	125,70 ± 22,70
LC_{50} (Lactose)	510,30 ± 127,30
Selectivity index the VML (IS)	**21,83 (> 7,96)**

380 **Table 2 - Selectivity index for the Trypomastigotes evolutionary forms of *Trypanosoma***
381 ***cruz*i.**

382

383 Based on the selectivity index, the VML lectin has a significant effect on the
384 evolutionary forms of Trypomastigotes. Considering the activity profile, other evolutionary
385 forms can be evaluated for lectin activity. Most of the compounds under evaluation refer to
386 small compounds, such as secondary metabolites such as flavonoids (OLIVEIRA et al., 2022)
387 or the repositioning of drugs, in an attempt to reposition other drugs already available on the

market. However, analysis of the technological market registered by patent applications demonstrate that the journey is still long until the arrival of these compounds to clinical trials and their future incorporation in the health care of patients with Chagas disease.

Glycans and Evaluation of interactions between GIPL and the VML lectin

GILP used in molecular docking

The glycans present in the plasma membrane of *T. cruzi*, modeled using the ChemSketch program, are represented in their three-dimensional structure in figure 8. The modeling of the glycans was necessary due to the absence of experimentally resolved three-dimensional structures or the availability of models in the databases public.

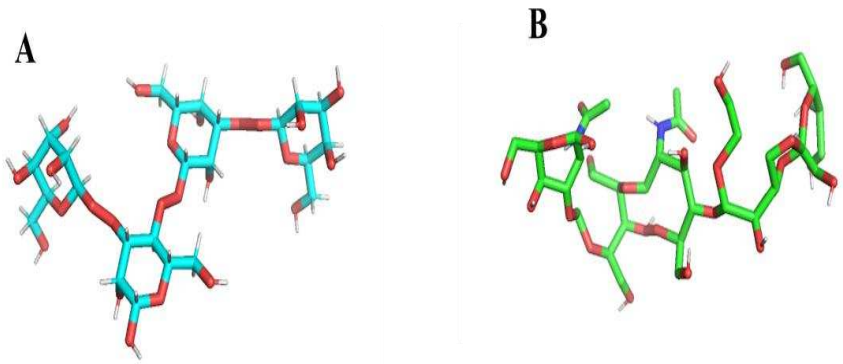


Figure 8. Glycoinositolphospholipid from *Trypanosoma cruzi*. A – LPG modeled according to data from Ferguson 1997; B – Gal-GalNAc-GlcNac saccharide.

Determination of the interaction between GIPL and the VML lectin

The VML lectin showed greater affinity for the saccharide GIPL when compared to the saccharide Gal-GalNAc-GlcNac as shown in table – 3. However, the molecule has better affinity for the disaccharide lactose.

Ligands	Residues	Energy Affinity (kcal/mol)
GIPL	Lis-102 Asn-129 Thr- 130 Asp- 216	-2.6
Trissacarídeo	Lys-102 Asn-129 Thr-130 Asp-216	3.5
Lactose	Leu213 Ser-214 Asn-129	-5.1

Table 3. Interactions between saccharides and VML lectin.

The redockink was used to validate the system, being able to reproduce the experimental data through the established parameters. Initially, the molecular anchoring of the VML and lactose was carried out, for which it was possible to position the molecule in the DRC of the lectin figure 9. Through the method, the interactions of the molecules with the integral residues of the carbohydrate recognition site of the protein were observed. The other complexes are illustrated in figures 10 and 11.

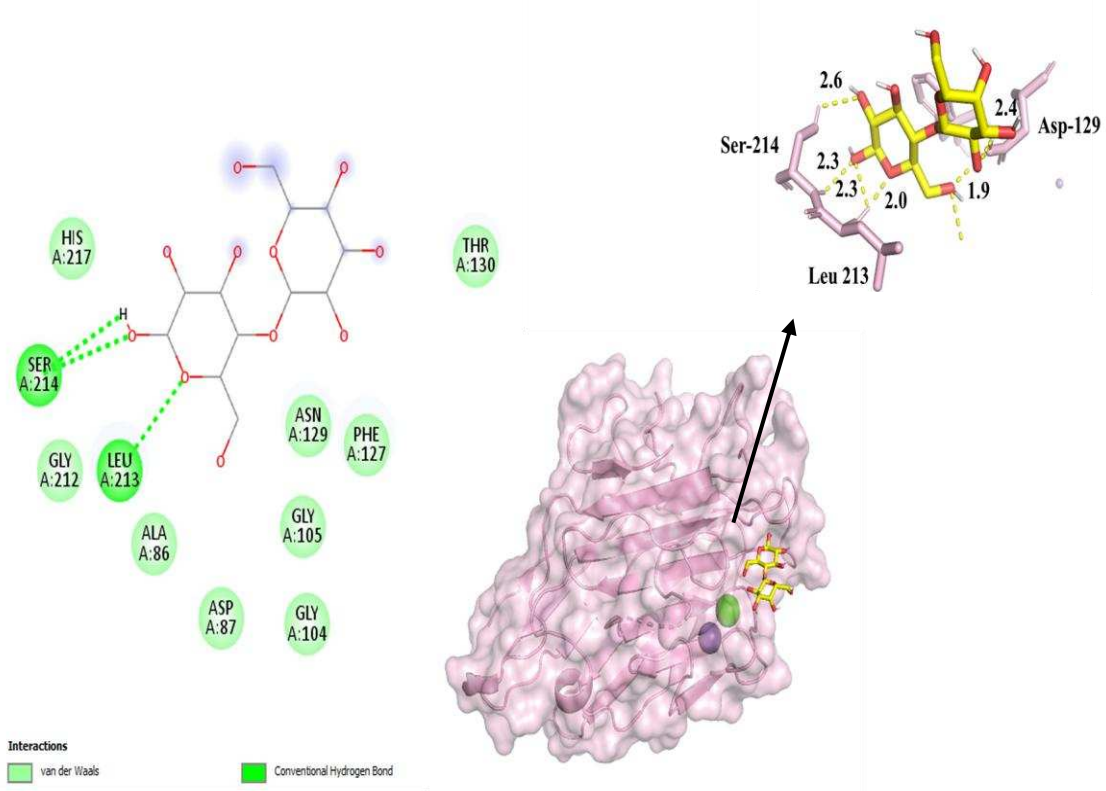


Figure 9 – Complex formed by interactions between the VML lectin and lactose

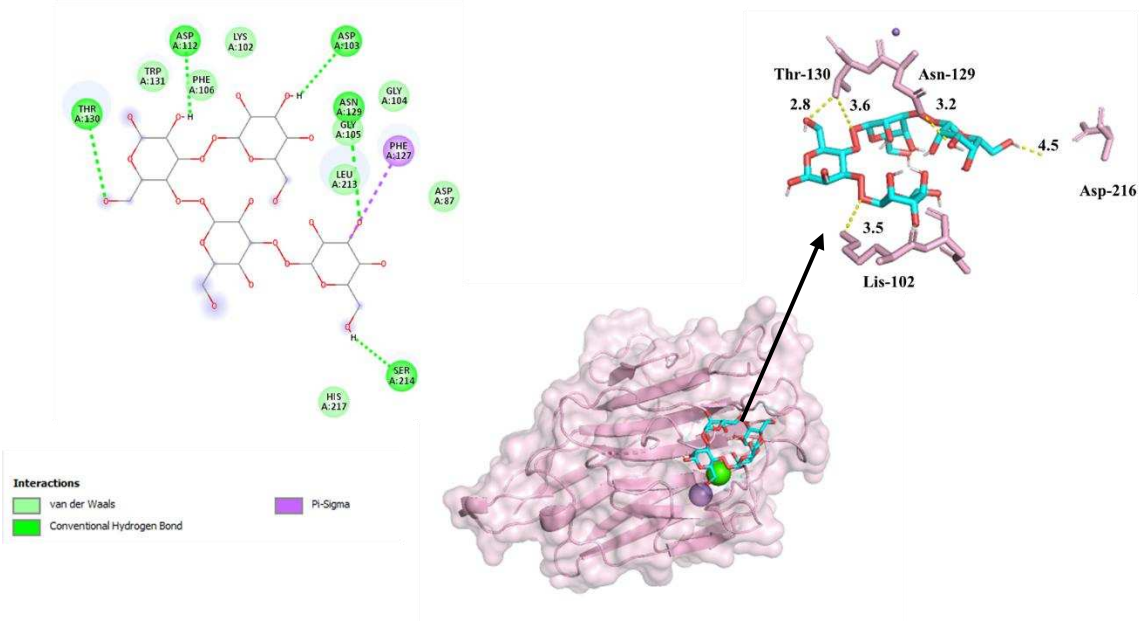


Figure 10 – Complex formed by the VML lectin and the GIPL saccharide

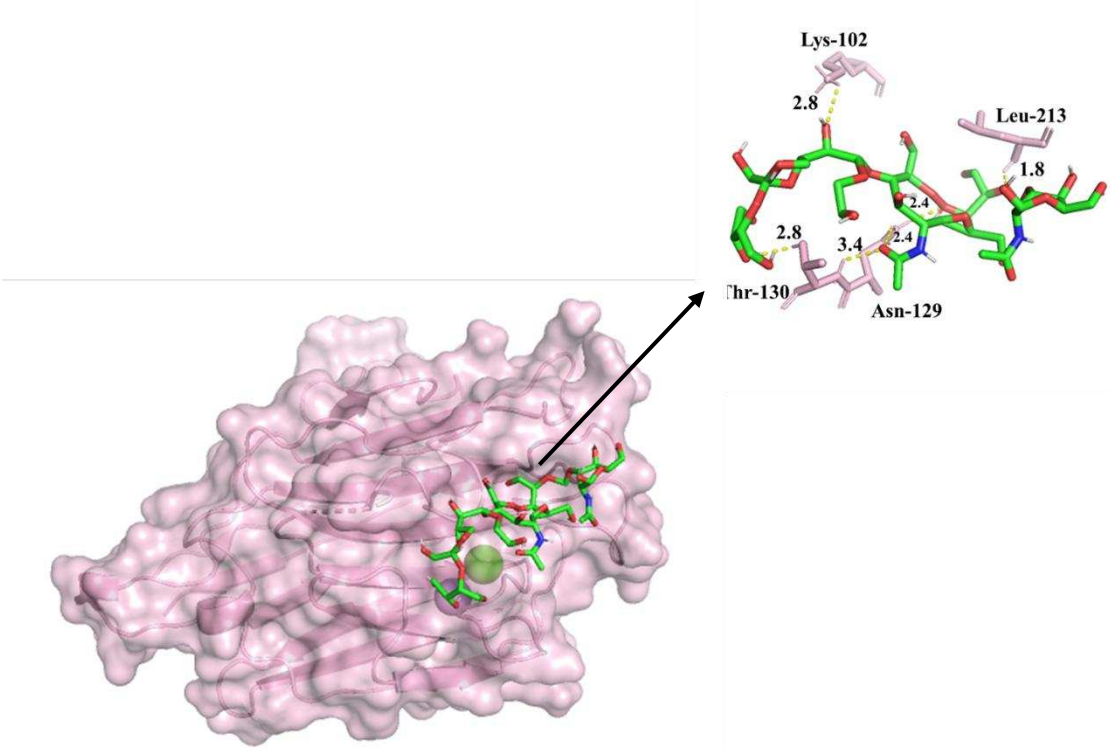


Figure 11 – Complex formed by the VML lectin and the Gal-GalNAc-GlcNac saccharide.

However, when carrying out the molecular anchoring with GIPL, a lower affinity energy for the molecule was verified, being then positioned in the region close to the DRC of the lectin. The difficulty in positioning the molecule in the site lies in the extension of the ligand and in the inherent flexibility of the molecule itself. Similar difficulties were observed in analyzes carried out by Sousa (2014) when trying to reproduce by molecular anchoring the interaction of the VML lectin and fragments of O-linked mucins. The observed difficulty can

be explained by the findings made by Bezerra (2011) who emphasize the relationship between the size of the protein binding site and the binding capacity of the legume lectin.

CONCLUSION

The A lectin showed trypanocidal activity for the trypomastigote evolutionary form and lack of cytotoxicity for host cells. The molecular anchors indicate that the interaction between the glycans present in the plasmatic membrane of the parasite occurs in the region of the carbohydrate recognition domain of the lectin, possibly being its action linked to the lectin domain of the protein.

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5 CONCLUSÃO

O estudo demonstrou a urgência da realização de pesquisas que investiguem o potencial de novas moléculas que possam ser incorporadas ao enfrentamento da doença de Chagas, dados os recursos limitados de formulações farmacêuticas atualmente disponíveis para tratar a doença, assim como o desenvolvimento de métodos acessíveis para o estabelecimento do diagnóstico precoce. A lectina apresentou atividade tripanocida para a forma evolutiva tripomastigota e ausência de citotoxicidade para as células hospedeiras. Os ancoramentos moleculares indicam que a interação entre os glicanos presentes na membrana plasmática do parasito ocorre na região do domínio de reconhecimento a carboidrato da lectina, estando possivelmente sua ação vinculada ao domínio lectínico da proteína.

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